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ANAEROBIC TREATMENT OF SOLID MANURE RESIDUES

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ABSTRACT

Spent bedding, which is a mix of bedding stubble, faeces, urine, spilt water and animal feed, is a major by-product of livestock farming in Australia and the United States. In Australia, such residues are stockpiled for passive composting prior to land spreading to harness the nutrient value. Key concerns are uncontrolled greenhouse gas emissions, odour, losses of nutrients, and the potential for ground and surface water contamination. A sustainable management alternative is anaerobic digestion (AD), whereby organic matter in the spent bedding is converted into useable methane energy and nutrients are mobilized for subsequent recovery. However, AD technology is not currently available in Australia at a sufficiently low cost and low level of complexity for on-farm adoption. In addition, the AD characteristics of solid manure residues are not well understood, posing significant uncertainty for AD design and optimization. In response, this thesis examines a leachbed AD approach, which is particularly suitable for high solids wastes such as spend bedding, and can be cost-effective and simple to construct and operate.

A pilot scale (200 L) leachbed system was constructed and operated. Spent bedding was collected from Australian piggeries and wetted with water leachate in the enclosed leachbed via two distinct operating modes: trickling and flood-and-drain. The water leachate was heated in both configurations, aimed at a 37 ^{o}C operating temperature. Results showed comparable methane recovery for both the trickling and flood-and-drain modes at 50% of the biochemical methane potential (B_0 = 195 - 218 L CH_4 kg VS_{fed}^{-1}) in both cases. This indicated that AD performance was insensitive to the mode of leachate flow. However, the flood-and-drain leachbed did mobilise more particulates into the leachate than the trickling leachbed, which could cause a materials-handling problem with pumping of leachate on-farm. Inoculation with solid digestate from a previous leachbed (inoculum-to-substrate ratio of 0.22 on a VS basis) hastened the AD start-up, but methane recovery remained at 50 % of the B_o. Post-digestion testing indicated that methane recovery was limited by insufficient indigenous inoculum and/or by chemical inhibition.

To clarify the effects of operating conditions on AD performance at high solids content, a series of batch tests were performed at smaller laboratory scale. These tested the viability of indigenous inoculum in spent bedding (from the manure component), together with the effects of operating temperature and solids concentration. Results showed that spent bedding actually did have sufficient indigenous inoculum to recover the full B_o of the spent bedding. However, to operate at higher solid loading (≥ 10 % TS content) and higher

temperatures (55 ºC), methanogenic activity could be boosted by inoculation with leachate and/or solid digestate from a former leachbed batch. The use of leachate as an inoculum would preserve leachbed volume for fresh waste and easier handling.

Chemical analysis of digestate revealed that leachbeds could have high concentrations of the chemical inhibitors ammonia and humic acid (HA). In the pilot trials ammonia concentration was below the inhibitory threshold for AD, so the focus of further study shifted to HA. Inhibition testing showed that hydrolysis was more prone to inhibition than methanogenesis at HA concentrations of 0 to 20 g L^{-1}. Also carbohydrate hydrolysis was more susceptible to HA inhibition than protein hydrolysis. The results suggested that at HA concentration below 5 g L^{-1}, hydrolysis inhibition was due to inactivation of hydrolytic enzymes by HA. However, beyond HA concentration of 5 g L^{-1}, AD seemed to be affected by more complex mechanisms. Inhibition appeared to be reversible, but recovery rate depended on hydrolytic activity. Increased HA inhibition resilience may result from higher microbial activity or microbial concentration, independent of differences in microbial community composition.

Overall, the study provided insight and certainty on leachbed performance factors for spent bedding. Likely future applications would include decentralized installations, with leachbeds operating in tandem with existing covered anaerobic ponds for enhanced methane recovery and water reuse.

Declaration by author

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Publications during candidature

Peer reviewed papers

Boyle-Gotla, A., Jensen, P.D., Yap, S.D., Pidou, M., Wang, Y., Bastone, D.J., 2014. Dynamic multidimensional modelling of submerged membrane bioreactor fouling. *Journal of Membrane Science*, **467**, 153-161.

Jensen, P.D., Yap, S.D., Boyle-Gotla, A., Janoschka, J., Carney, C., Pidou, M., Batstone, D.J., 2015. Anaerobic membrane bioreactors enable high rate treatment of slaughterhouse wastewater. **97**, 132-141.

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Contributor	Statement of contribution
Yap, S.D. (candidate)	Conducted experiments (100%) Designed experiments (60%) Wrote the paper (60%)
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Jensen, P.D.	Designed experiments (5%) Wrote and edited paper (5%)
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Anaerobic digestion, leachbed, solid-phase, manure, straw, inoculum, indigenous microbial community, humic acid, inhibition, hydrolysis.

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Acronyms

AD	Anaerobic digestion
BMP	Biochemical methane potential
CH_4	Methane gas
CO_2	Carbon dioxide gas
COD	Chemical oxygen demand
DOM	Dissolved organic matter
EEM	Excitation emission matrix
FIA	Flow injection analysis
FID	Flame ionization detector
GC	Gas chromatography
GHG	Greenhouse gas
H_2	Hydrogen gas
HA	Humic acid
ISR	Inoculum to substrate ratio
N_2	Nitrogen gas
N_2O	Nitrous oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NH_3	Free ammonia
OFMSW	Organic fraction of municipal solid waste
OTU	Operational taxonomic unit
PCA	Principal component analysis
PLC	Programmable logic control
PO_4^{3-}	Phosphate
RTD	Resistance temperature detector
SMA	Specific methanogenic activity
SS	Stainless steel
sCOD	Soluble COD
TCD	Thermal conductivity detector
TS	Total solids
TAN	Total ammoniacal-nitrogen

UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acid
tCOD	Total COD
VS	Volatile solids

Nomenclature

B_0	Maximum potential methane yield (L CH_4 kg VS^{-1})
f_d	Degradability extent (%)
K_a	The acid-base equilibrium coefficient
k	Apparent first-order kinetic rate coefficient (d^{-1})
k_{hyd}	First order kinetic of hydrolysis (d^{-1})
k_{meth}	First-order kinetic of methanogensis (d^{-1})
r	Degradation rate of substrate (g COD g COD^{-1} d^{-1})
t_d	Time delay (d)
T	Temperature (˚C)
X	Particulate component (g COD g COD^{-1})
S	Soluble component (g COD g COD^{-1})

CHAPTER 1
Introduction

This chapter outlines the motivation for the thesis research and concludes with stated research objectives and an overview of the thesis approach.

1.1 Research motivation

Livestock industries produce increasing amounts of solid manure residues (Menzi et al., 2010). In Australia alone, the livestock production systems could produce more than three million tonnes of manure each year (Tromp, 2012). Solid manure residues typically consist of a fibrous and lignocellulosic base bedding material on which the livestock is reared, mixed with faeces, urine, spilt feed and water from drinkers progressively deposited as the livestock grow. This manure residue is often regarded as a valuable source of nitrogen, phosphorus, trace elements and carbon to support crop growth (Mafongoya et al, 2000). However, typically manure residues are first stockpiled to passively compost before being applied to land as a fertilizer/soil conditioner, resulting in uncontrolled greenhouse gas (GHG) emissions (N_2O and CH_4) (Wiedemann, et al., 2016), and an increased risk of pest propagation, dust and odor nuisance (Sommer and Møller, 2000), and land and water contamination.

Treatment of solid manure residues with anaerobic digestion (AD) technology offers significant advantages over conventional stockpiling, including renewable energy generation in the form of biogas and mobilizing nutrients for subsequent recovery. Biogas and fertilizer outputs could provide financial incentive for adoption of AD technology, and could reduce GHG emissions by displacing fossil-fuel derived energy and nutrient fertilizers (Wiedemann, et al., 2016). However, the complexity and cost of AD technology currently limits its adoption on-farm, especially in Australia (Wilkinson et al., 2011).

Solid-phase anaerobic leachbeds is a type of batch anaerobic digester that is relatively simple to construct and operate. Therefore, leachbeds could be much more cost-effective than alternate AD technologies such as continuous solid-phase or mixed liquid slurry digesters (Deublein and Steinhauser, 2011). The leachbed process involves intermittent wetting of a bed of solid waste to initiate and support the AD process until the waste is stabilised. Leachbeds can operate at relatively high solids content (>20%) (Batstone and

Jensen, 2011), making them particularly interesting for high-solids substrates such as solid manure residues. Leachbeds can also conserve water compared to liquid-phase AD systems and eases materials-handling for solid digestion substrates. However, leachbeds have been mostly used for the organic fraction of municipal solid waste (OFMSW) and energy crops (Vandevivere et al., 2003; Deublein and Steinhauser, 2011), and minimal studies have investigated their use with solid manure residues (Vandevivere et al., 2003; Deublein and Steinhauser, 2011). In general, leachbeds have been prone to relatively poor performance with low methane yield (Kusch et al., 2008; Lehtomaki et al., 2008; Xu et al., 2011; Browne et al., 2013). There appears to be considerable opportunity to improve the performance of leachbeds via systematic research and development.

Mass transfer is a key parameter that can influence the performance of leachbeds. Good contact between leachate and the solid being digested is essential, in order to provide dispersion of inoculum, nutrients, soluble digestion products and pH buffering agents (Jha et al., 2011). In an attempt to improve mass transfer and so AD performance, past studies have trialled different leachate recirculation modes to enhance the solid-liquid contact (Chugh et al., 1988; Kusch et al, 2008; Nizami et al, 2010). In some cases, leachate is sprayed over the solid bed of material being digested and the leachate recovered from the base of the leachbed for recirculation (termed trickling) (Deublein and Steinhauser, 2011). In other cases, the solid bed is fully flooded with leachate (Nizami et al., 2010), left flooded for a period to contact, and then drained (termed flood-and-drain) (Clarke and Xie, 2013). The preferred mode of leachate contact is still unknown (Kusch et al., 2008; Nizami et al., 2010) because previous studies have not compared different leachate flow configurations in parallel for the same waste substrate, and performance and preferences may be specific for each waste type. Therefore, performance differences need to be clarified for the substrate of interest in this thesis, namely solid manure residues.

Typically, an inoculum source is required for a balanced microbial community during leachbed start-up (Batstone and Jensen, 2011). Previous studies have examined the indigenous microbial community on solid manure residues as a source of self-inoculation (Kusch et al., 2008; Tait et al., 2009), because an external inoculum may not be readily available within the vicinity of a farm. In Australia, many farms are very remote and have stringent biosecurity restrictions limiting the flow of materials onto farms. Also, self-inoculation would be of benefit, because the addition of an external inoculum would reduce available AD capacity for fresh substrate. Previous studies have suggested that indigenous

microbial communities in solid manure residues could be capable of starting up a digester (Kusch et al., 2008; Tait et al., 2009), but did not determine the microbial proficiency to recover the full methane potential of the substrate. Limited understanding of indigenous microbial response to different operating conditions in leachbed, could further impede progress in this field. Indigenous microbial capability in solid manure residues thus merit further investigation, as it could lead to more effective inoculation strategies that accelerate leachbed start-up and digestion.

As a leachbed process is operating with high solids content, inhibitors and/or toxicants could accumulate up to substantial concentrations within the system, thus affecting digestion efficiency (Motte et al., 2013). For example, a manure digester was known to be inhibited by ammonia (Yenigun et al. 2013) and/or humic acid (HA) (Brons et al., 1985; Fernandes et al., 2014), which is considered highly relevant for solid manure residues. Yet relevant studies have largely focused on the inhibitory effect of ammonia (Yenigun et al. 2013) with much less attention given to HA (Brons et al., 1985; Fernandes et al., 2014). This is despite the observations of previous works that suggested that HA could inhibit substrate solubilisation (Brons et al., 1985; Fernandes et al., 2014). Prior studies have focussed on physico-chemical methods to mitigate HA inhibition, such as precipitation or complexing with metal salts (Azman et al., 2015; Brons et al., 1985) or ion exchange (Boyer and Singer, 2006; Fearing et al., 2004). However, such methods are unlikely to be cost effective with AD of agricultural residues. It is of interest to identify links between AD performance and microbial activity and community composition, to determine whether inoculation with resilient communities could promote HA inhibition resilience and thus enhance AD.

1.2 Research aim, objectives and approach

This PhD aims to understand leachbeds as a potential anaerobic technology option for solid manure residues, in order to optimise processing into biogas. Specific objectives of the PhD study were:

- Research objective 1: Quantify the effect of leachate recirculation mode on digestion performance of solid manure residues in a leachbed;

To address this objective, Chapter 4 describes leachbed trials at 200 L pilot-scale that were conducted to examine the impact of leachate flow configuration and inoculation on digestion performance with solid manure residues.

- Research objective 2: Quantify the influence of various operating conditions on solid-phase digestion performance with solid manure residues;

  This objective is addressed in Chapter 5 through batch anaerobic digestion experiments at smaller laboratory scale, evaluating the effect of initial solids concentration, temperature and inoculation techniques on start-up and AD performance with solid manure residues.

- Research objective 3: Quantify and better understand the inhibition potential and inhibition mechanisms of humic acid (HA).

  This objective is addressed in Chapter 6 via batch activity tests at smaller laboratory scale that measured the inhibitory effect of HA on AD of model substrates (cellulose, gelatin and acetate) for various distinct inocula. This work examined microbial capability as a potential means to overcome HA inhibition.

CHAPTER 2

Literature review

This chapter reviews the pertinent literature relevant to the thesis, highlighting key knowledge gaps. Key concepts are also introduced, including solid manure residues, anaerobic digestion (AD) in general and specifically solid-phase AD in a leachbed.

2.1 Solid Manure Residues

Livestock industries produce increasing amounts of solid manure residues (Menzi et al., 2010). The specific type of solid manure residue considered in this thesis is spent bedding, consisting of a fibrous and lignocellulosic base bedding material such as straw, saw dust or rice husks on which the livestock is reared, mixed with faeces, urine, spilt feed and water from drinkers progressively deposited as the livestock grow. Characteristics of spent bedding vary significantly from farm to farm (Table 2.1). This is due to different base bedding materials used, differences in feed intake and diets, differences in the size / class of livestock being reared (e.g. for pigs this could be weaners, growers or finishers) and extent of soiling (Gopalan et al., 2013). The extent of soilage, which indicates the manure content, is influenced by the rate of fresh bedding added to the shed, stocking density and the length of time the bedding is exposed to the livestock.

Table 2.1. Spent bedding characteristic

Reference	**Parameters**					
	Livestock class	**Total solid (TS, %)**	**Volatile solid (VS, %)**	**Total ammonia (g-N kg^{-1})**	**Volatile fatty acid (VFA, g kg^{-1})**	**Methane yield (mL CH_4 g VS $^{-1}$)**
Tait et al. (2009)	Pig	20 - 80	20 - 80	1.1 – 7.5	0.1 - 40	
Cui et al. (2011)	Horse	94	89	n/a	11.4	100-150
Li et al. (2013)	Poultry	25	19	2.8	0.5 – 1.3	480
Wang et al. (2013)	Poultry	25	16	0.2	n/a	170
Borowski et al. (2014)	Poultry	28	21	n/a	n/a	n/a

Solid manure residues can be produced in batches as the livestock are reared according to a batch “all in, all out” mode (Tucker et al., 2010). This operating mode involves moving pigs of similar age and weight into a phase of production (and a dedicated shed) together as a whole group according to the production schedule. When a group is moved on, the spent bedding is emptied from the pig shed in a large batch of material. The batch time could be between 4 to 6 weeks depending on the size / class of livestock being reared (Tucker et al., 2010).

Generally, solid manure residues are stockpiled for passive composting prior to spreading onto land as a fertilizer or soil conditioner (Wiedemann, 2016). With this current management system, there is very little control over emissions of the GHG from the composting during stockpiling (Wiedemann, 2016). Direct application of solid manure residue to land, poses risk of nutrient leaching through the soil profile or losses via surface runoff and eroded soil. Long-term monitoring of soil quality may be necessary which would increase management input and therefore production costs. Other potential issues include odour and dust nuisance, propagation of pathogens, and breeding of rodents, flies and other pests (Sommer and Møller, 2000). There is a clear need to consider alternative strategies for management of solid manure residues and technologies to resolve environmental challenges posed by conventional manure management practices.

Anaerobic digestion (AD) is a mature technology and has been widely used to treat various organic wastes (Mata-Alvarez et al., 2000). AD is a natural biological process that progressively breaks down organic matter into an inert nutrient-rich residue (digestate) and biogas (CH_4/CO_2), in the absence of oxygen (“anaerobic” meaning no air/oxygen). For solid manure residues, the benefits of AD over conventional waste management practices include (Deublein and Steinhauser, 2011):

- capturing and preventing GHG emissions;
- a high degree of waste stabilization;
- destruction of pathogens;
- generation of renewable energy (biogas), offering superior energy efficiency over other waste treatment alternatives; and
- nutrient recovery for beneficial reuse.

Key considerations with AD technology selection and design with solid manure residues include:

- Solid manure residues are often produced in batches and have high solids content (> 40% TS concentration) (Kusch et al., 2008; Tait et al., 2009);
- Solid manure residues contain essential nutrients suitable for crop growth (Kruger et al., 2006), but also contain ammonia, which can be both an inhibitor and pH buffering agent in AD (Yenigun et al. 2013);
- The manure component of solid manure residues could provide indigenous microbial activity for inoculation and start-up of AD (Kusch et al., 2008; Tait et al., 2009);
- The base bedding materials in solid manure residues is an inherent “bulking agent” that make the residue stackable (Staub et al., 2009), ideal for leachbed systems as discussed below;
- Base bedding materials provide additional carbon and complements the manure component for methane production; and
- Solid manure residues with high lignin-cellulose material could degrade slowly, likely requiring a minimum solids retention time for AD between 30 to 50 days (Batsone and Jensen, 2011).

2.2 Anaerobic digestion - Biological principles

AD is a complex microbiological process, mediated by three broad functional groups of microorganisms, namely; acidogenic bacteria, acetogenic bacteria and methanogenic archaea. While the majority of the bacterial population are obligate anaerobes, there are also facultative microbes (Birkett and Lester, 1999). AD proceeds via a series of parallel and sequential biological reaction steps as shown in Figure 2.1, which includes hydrolysis, acidogenesis, acetogenesis and methanogenesis.

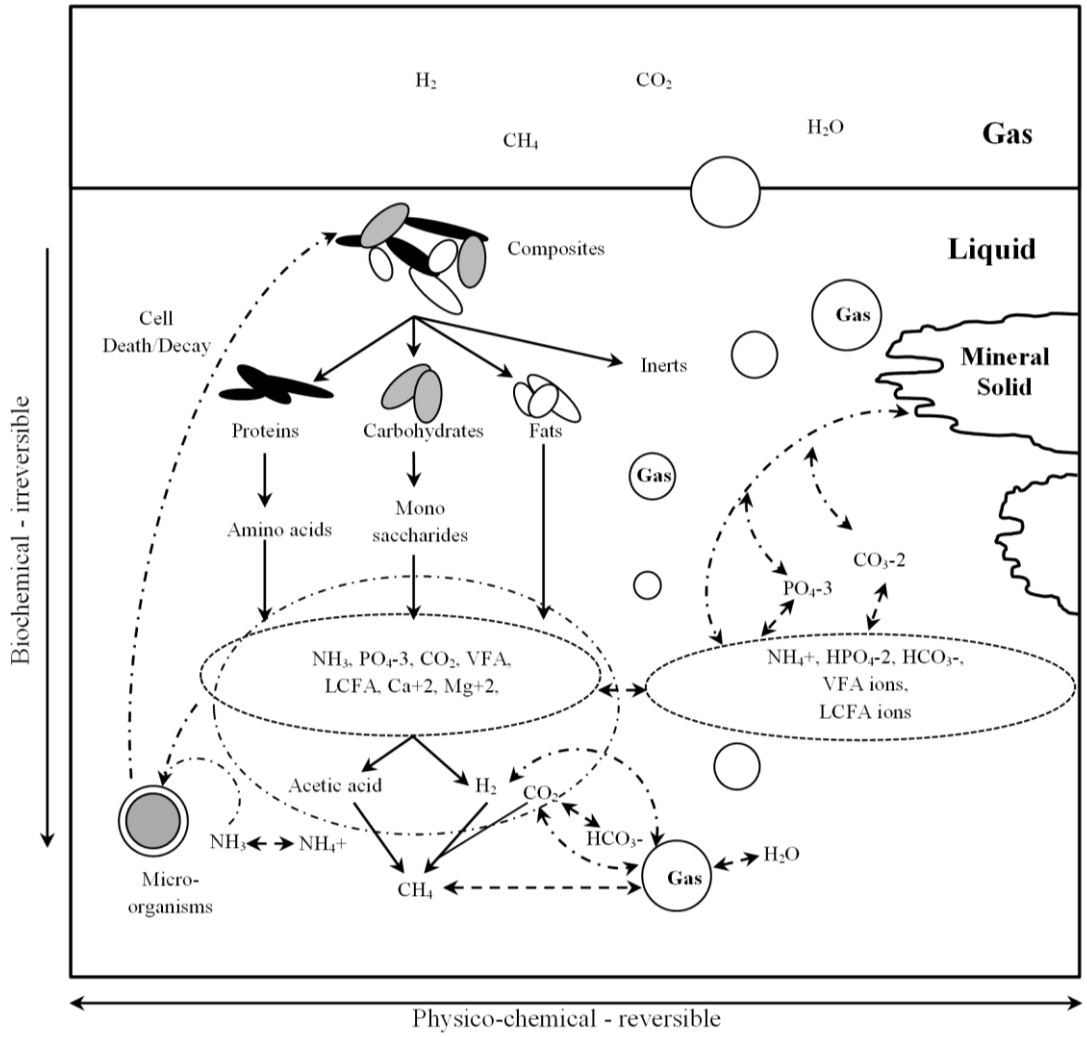

Figure 2.1. Anaerobic process key step (Batstone and Jensen, 2011).

2.2.1 Hydrolysis

Hydrolysis, an enzyme-mediated extracellular process, involves solubilization of complex particulate organic matter into soluble substrates. The organic matter typically consists of carbohydrates, proteins and lipids. The enzymatic degradation products of carbohydrates, proteins and lipids are monosaccharides, amino acids and long chain fatty acids, respectively. Possible mechanisms involved in enzymatic hydrolysis are (Batstone et al., 2002):

- Organisms produce enzymes into the bulk liquid where the enzymes adsorb onto a particle substrate or react with soluble substrate (Figure 2.2), and/or

- The organism attaches to the particulate matter and excretes enzymes that hydrolyse the particulates and the organism directly harnesses the soluble substrates that are released by the hydrolysis, and/or

- The organism with an attached enzyme on its surface that act as transport receptor to the interior of the cell. This mechanism requires the organism to attach onto the particle's surface.

Hydrolytic bacteria are commonly, though not exclusively, *Enterobacteriaceae* and the genera of *Clostridium*, *Bacteroides*, *Eubacterium, Paenibacillus,* and *Ruminococcus* (Deublein and Steinhauser, 2011; Lauwers et al., 2013).

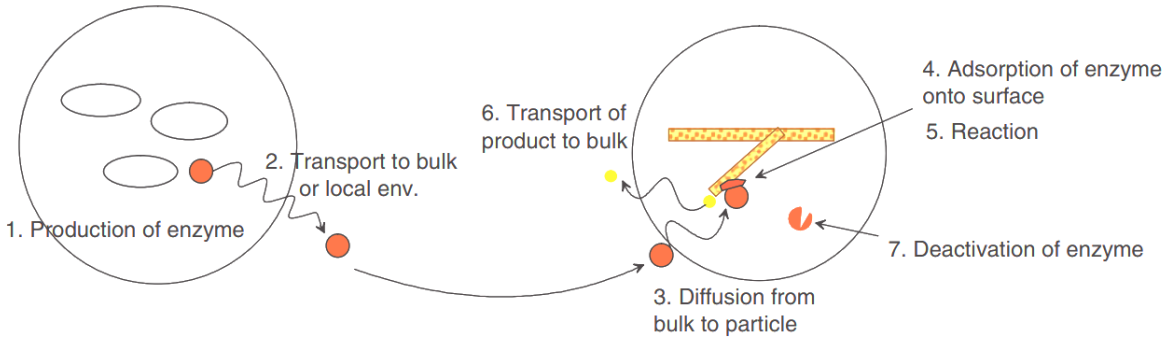

Figure 2.2. Possible enzymatic steps for substrate hydrolysis in the bulk liquid phase (Batstone and Jensen, 2011).

2.2.2 Acidogenesis/ Fermentation

This process converts soluble substrates such as amino acids and sugars into VFAs, alcohols, acetate, CO_2 and hydrogen. These processes are conducted by a complex consortium of hydrolytic and non-hydrolytic microorganisms. Nearly all acidogenic microorganisms also participate in hydrolysis (Deublein and Steinhauser, 2011).

2.2.3 Acetogenesis

In this process simple organic acids and alcohols are degraded into acetate, CO_2 and hydrogen by anaerobic oxidation. Organic acids are degraded into acetate or propionate via beta-oxidation (Batstone et al., 2002). Specialized oxidising organisms are required to convert propionate into acetate. Alcohols are degraded into acetate via acetyl-CoA

(Batstone et al., 2002). This process utilises hydrogen ions or bicarbonate as additional electron acceptor, which produces hydrogen gas or formate, respectively (Batstone et al., 2002). The thermodynamics of the oxidation reaction requires the concentration of the electron carriers (hydrogen partial pressure) to be low and therefore acetogenesis is obligately linked to hydrogen utilisers, such as hydrogenotrophic methanogens (Batstone et al., 2002). In this, acetogenic bacteria can only survive in symbiosis with hydrogen consuming or formate-removing species, which can be hydrogen utilizing archaea or sulphate reducing archaea or bacteria (Wheatley, 1990).

2.2.4 Methanogenesis

This is a biological process during which acetate is converted into CH_4 and CO_2 by methanogens, either by an aceticlastic reaction or by an anaerobic acetate-oxidizing reaction (Batstone et al., 2002). Another small portion of CH_4 is produced from the oxidation of molecular hydrogen (or formate (HCOOH)) using CO_2 as an electron acceptor. Generally, 60-70 % of the CH_4 originates from acetate cleaving, with the remainder produced from hydrogen and CO_2. Methanogenesis is dominated by methanogenic Archaea. *Methanobacterium*, *Methanosirillum hingatii*, *Mehanosarcina* and *Methanosaeta* are common species (Deublein and Steinhauser, 2011; Lauwers et al., 2013).

2.2.5 Rate limiting step in AD

Due to the nature of solid manure residues, the AD process rate is likely limited by (1) hydrolysis, due to presence of lignocellulosic material and mass transfer limitations, and/or (2) methanogenesis, which can be particularly sensitive to chemical inhibition (Vavilin et al., 2008). Presence of microbial community on solid manure residues affects substrate degradability and digestion rate, and thus the reactor size required for AD. Further, the AD rate to some degree is influenced by the configuration of the digester. The relevant digestion technology options for solid manure residues are outlined in section 2.3.

2.3 Anaerobic digestion technology options for solid manure residues

AD is a mature waste treatment technology, including for the treatment of municipal solid wastes, market place wastes and industrial wastes (De Baere, 2000). For context, the present section emphasizes AD technology options for solid manure residues, specifically for on-farm agricultural applications.

AD technologies can be generally classed as continuous or batch. With a continuous system, the waste is fed semi-continuously or continuously, while at the same time removing digestate. In a wet continuous system, the organic solid waste is diluted with water into a pulp or slurry with TS concentrations less than 15 %, so that a completely stirred digester can be used (Vandevivere et al., 2003). A major drawback of the continuous wet system is high water consumption necessary to dilute the solid waste, which could incur high financial costs for water purchases, treatment before disposal and possible discharge. This also means larger reactors and operating units to accommodate several-fold increases in waste volume (Vandevivere et al., 2003). In a dry continuous system, on the other hand, the TS concentration within the reactor is kept in the range 20 - 30 % (Vandevivere et al., 2003). Due to high viscosity, the solid waste in a dry system moves via plug flow inside the reactor. With the dry continuous system, there is a need for mixing the incoming wastes with the digested waste, which is crucial to guarantee adequate inoculation and prevent local overloading and acidification hotspots downstream in the digester. Continuous systems are less likely to be applicable for treating solid manure residues at farm-scale, due to technical complexity and high investment cost. Continuous systems will generally be more feasible for large and centralized installations.

In a batch system (also known as a leachbed), the digester is filled once with fresh waste, and then sealed to allow the waste to stabilize in a semi-dry mode (> 30% TS). Batch systems do not require any in-vessel mixing, but do rely on convective movement of a liquid (leachate) through the solid matrix for mass transfer and inoculation. The hallmark of the batch system is the separation between solid and liquid phase, which permits high spatial solids loading. Most of the leachate can be recovered at the end of each batch and re-used for a subsequent digestion batch, thereby minimizing water use. This type of technology offers integrated treatment options with nutrient recovery from leachate (Batstone and Jensen, 2011; Vandevivere et al., 2003) and/or with available on-farm infrastructure such as an existing covered anaerobic lagoon. There are three basic leachbed designs, which

differ in terms of the respective locations of hydrolysis and methanogenesis phases. These are illustrated in Figure 2.3:

- In single stage batch system, the leachate is recirculated via the same reactor, where biogas is produced until the solid waste is stabilized;
- In the sequential batch design, the leachate of a freshly-filled reactor, containing high levels of organic acids, is recirculated to another mature reactor where methanogenesis takes place; and
- For hybrid batch-UASB, the design is similar to a dual stage system operating in batch, with the difference being that the first stage is a simple fill-and-draw leach bed reactor instead of a fully mixed design and the second stage is replaced by an upflow anaerobic sludge blanket (UASB) reactor. The UASB reactor, wherein anaerobic microflora accumulate as granules, is well-suited to treat liquid effluents with high levels of organic acids at high loading rates (Chen et al., 2008).

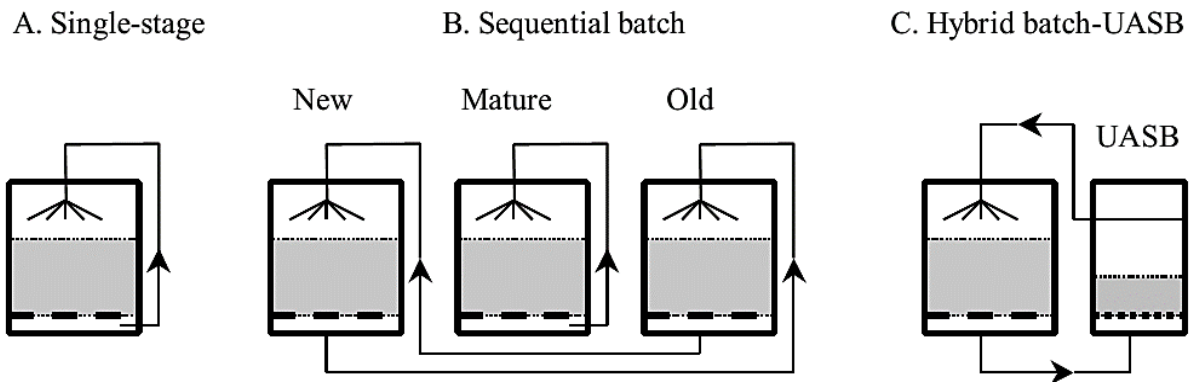

Figure 2.3. Configuration of leachate recirculation patterns in different leach bed system (Vandevivere et al., 2003).

One technical shortcoming of leachbeds is the potential for clogging, channeling and short-circuiting of leachate flow, also causing issues with leachate recirculation and wetting of the solid bed. These are of particular concern for larger scale systems (Mussoline, 2013). Batch systems also give variable biogas flow and composition, due to the batch operation. To provide more consistent biogas production, multiple leachbeds can be setup and operated in parallel as single stage, or fed to a single methanogenic reactor in dual stage configuration

(Nizami et al., 2010; Browne et al, 2013). Safety measures need to be closely observed during the loading and emptying of the batches, because personnel performing the unloading/loading could be exposed to hazardous conditions. For these reasons, the loading and unloading steps of batch digestion can be more complex and labour-intensive.

However, specifically for solid manure residues, leachbeds:

- have the capability to achieve high solids and space loading, with solid manure residues being stackable without the need for a bulking agent;
- allow the substrate to be largely handled in the original solid state, without requiring any pre-treatment (chopping or macerating) or conditioning (slurrying up), reducing system capital and operating costs and complexity and materials handling;
- are flexible and appropriate for a farm that has intermittent waste production, such as with all-in-all out livestock production systems (See Section 2.1 above); and
- enable efficient water-reuse, because of the recovery and reuse of leachate.

Therefore, despite the above-listed draw-backs of leachbeds, the relatively simple design and operation of batch leachbeds, robustness towards coarse matter and high-solids and lower investment cost for decentralized installations, make them attractive for farm-scale AD of solid manure residues. Batch systems are also readily scalable to sizes typically encountered in farming businesses.

Unfortunately, the performance of leachbeds have been often suffer from inhibition problems due to lack of control (Jha et al., , 2011), resulting in only 20 - 30 % of methane recovery (Kusch et al., 2008; Lehtomaki et al., 2008; Xu et al., 2011; Browne et al., 2013). This results in large digester sizes and low biogas yield, affecting technical and economic feasibility of the leachbed technology. In recent years, significant efforts have been dedicated to improving batch AD performance with kitchen waste, agricultural waste and OFMSW (Vandevivere et al., 2003; Deublein and Steinhauser, 2011). Key influential factors identified include mass transfer limitations, microbial capability and chemical inhibitors. These factors are outlined in the sections that follow, specifically aiming to identify opportunities to better understand, manipulate and optimize AD performance with solid manure residues.

2.4 Batch solid-phase AD of solid manure residues, specific considerations

2.4.1 Leachate contact

Water is essential for mass transfer in leachbeds, to induce, support and maintain the AD microbiology, to transport soluble substrates of hydrolysis, and to dilute inhibitors to limit their impact. The motion of flushing the solid bed with liquid accelerates mass transfer by adding convective transport mechanisms to molecular diffusion. This promotes better solid degradation and higher methane yields owing to more efficient dispersion of inoculum, nutrients, buffering agents and enzymes required for the solid degradation (Bilgili et al., 2012; Stabnikova et al., 2008; Zuo et al., 2014). In a batch system, the moisture in the solid bed is typically replenished via sprinkling or flooding of the solid bed. With trickling, liquid is sprayed from a height onto the solid bed and allow trickling through the solid bed. With flooding, the solid waste is submerged in the leachate. Most published studies employ trickling as the means to replenish moisture in the solid bed. However, solid manure residues typically have a low wet shear strength, tend to collapse due to unidirectional downward flow of liquid under gravity, and can thus lead to exacerbated issues with clogging and leachate channeling (Staub et al., 2009, Figure 2.4). With trickling systems, an increase in leachate recirculation rate can increase mass transfer (Vavilin et al.,2002), thus improving AD rate (Chugh et al., 1998; Veeken and Hamelers, 2000; Vavilin et al., 2002). However, increased recirculation rates may not resolve leachate channelling (Morris et al., 2003). On the other hand, a flooded system enables the solid bed to be fully submersed with more intimate contact with leachate (Nizami et al., 2010). Also, leachate recirculation in varying flow direction could improve the porosity of a solid bed, thus minimizing clogging and leachate channeling (Uke and Stentiford, 2013). However, there is currently no consensus as to the preferred leachbed flow arrangement for leachbed digestion (Kusch et al., 2008; Nizami et al., 2010) and this requires further work as outlined in Section 2.5 below.

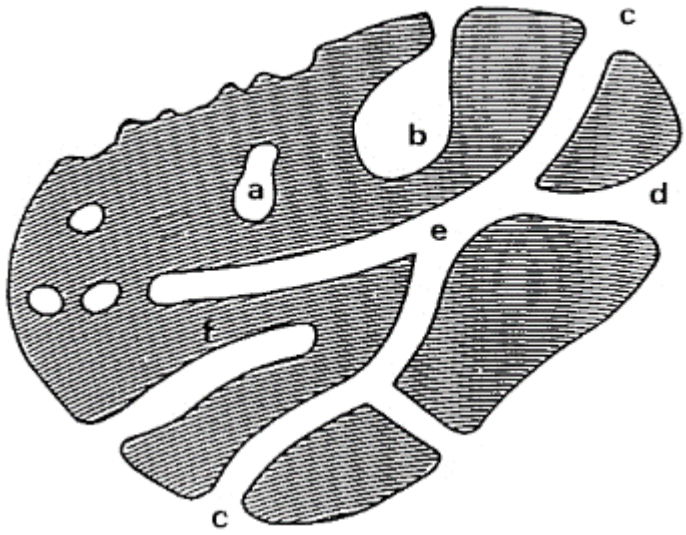

Figure 2.4. Schematic cross-section of a porous solid (Rouquerol et al., 1994). *(a)* is a *closed pore* and the remaining *(b)*, *(c)*, *(d)*, *(e) and (f)* are *open pores*. During leachate recirculation, open pores allow leachate to permeate and flow through the solid matrix. When solid waste becomes compact under gravity, the solid bed becomes impermeable as *open pore* networks collapse, which then leads to leachate channeling and clogging of the solid bed. Formation of *(a)* could trap leachate, causing less leachate water to be available for recirculation

2.4.2 Inoculation and microbial capability

Inoculation is often required for start-up to provide buffering capacity and serve as source of nutrients to establish a balanced microbial population to prevent a pH imbalance. A small number of studies have examined the indigenous microbial community in solid manure residue, in view of using the residue as a self-inoculant for AD (Kusch et al., 2008; Tait et al., 2009). The microbial composition and abundance on solid manure residues would be different from the shed to onsite manure storage due to different environmental conditions (Whiteley, et al., 2012). Nevertheless, the findings suggested that self-inoculation was possible, but it remains unknown whether solid manure residues contain adequate microbial presence and species to achieve full methane recovery from the substrate. From an applications perspective, there is limited knowledge on the response of the indigenous microbial communities to different histories (Whiteley, et al., 2012) and subsequent operating conditions in the leachbed. Such conditions include solids loading (also linked to inhibitor concentrations) and temperature. For example, leachbeds may operate at

thermophilic conditions for part of their batch life (Pietsch, 2014). That is, when pre-aeration is applied during start-up, temperature is increased by the heat released from pre-composting, thereby reducing the requirements for external heating (Kusch et al., 2008). The temperature in a batch system is commonly maintained via recirculation of warm leachate. The uniformity of temperature across the leachbed is highly dependent on leachate recirculation mode and frequency. The resulting impacts on overall digestion performance has been documented to some extent. For example, AD rate can be higher at thermophilic (55-70^{o}C) than at mesophilic conditions (35-37^{o}C) (De la Rubia et al., 2012; Fernández-Rodríguez et al., 2013). This may be related to increased microbial activity and growth rate at thermophilic temperatures (De la Rubia et al., 2012; Fernández-Rodríguez et al., 2013). However, there have been contradicting reports on the stability of start-up and operation of thermophilic digesters (De la Rubia et al., 2012; Hegde and Pullammanappallil, 2007). Specifically, the start-up of a thermophilic digester can be constrained by a lack of acclimated microbes, leading to process instability and higher risk of failure (De la Rubia et al., 2012).

Once the leachbed batch digestion has been completed, a fraction of solid digestate is typically retained to inoculate fresh waste added for the next cycle of treatment (Cysneiros et al., 2012). However, the use of solid digestate (1) reduces the treatment capacity for fresh solid waste and (2) could increase the tendency for clogging and leachate channeling. Alternatively, ‘sequencing’ or ‘indirect recirculation’ of leachate can be considered (Chugh et al., 1999), whereby leachate is exchanged between a batch of fresh waste and an older mature leachbed, and has been used extensively in practice for start-up of batch AD treating municipal solid waste. Studies by Jensen (2008) have indicated that reactors using this type of inoculum is biomass limited, due to low biomass concentration in leachate. Other previous studies (Nopharatana, 1999; Silvey et al., 2000; Lai. 2001) have suggested that successful start-up of batch systems via ‘sequencing’ was due to the presence of both indigenous microbial community on fresh solid waste and sufficient alkalinity (pH buffer) in leachate, rather than biomass concentration in leachate. The presence of alkalinity in AD prevents a rapid drop in pH due to accumulation VFAs, providing favorable environmental conditions for indigenous microbial activity to initiate AD. Increases in nitrogen loading to the digester, such as by the presence of manure, increases the release of ammonia which can facilitate with pH buffering (Nopharatana, 1999; Zhang et al., 2005). However, high ammonia can have a negative effect on methane production, because it is a well-known inhibitor (See Section 2.4.3 below).

2.4.3 Inhibition

pH inhibition is common in leachbeds, due to a high spatial solids/organic loading (Batstone and Jensen, 2011). At pH lower than 6, methanogens are strongly inhibited (Chen et al., 2008; Demirel and Scherer, 2008; Sträuber et al., 2012). The production of organic acids leads to a further decrease in pH by fermentative bacteria and can ultimately lead to the cessation of hydrolysis and acidogenesis by product feedback inhibition (Pratt et al., 2012). On the other hand, an increase in pH increases ammonia inhibition by a shift to a higher proportion of the more toxic form, namely free ammonia. Process instability due to ammonia often causes VFA accumulation, again leading to a decrease in pH with the effect of reducing free ammonia and inhibition (Chen et al., 2008). The interactions between free ammonia, VFA and pH may lead to a partially inhibited steady state, a condition where the process is operating stable but with lower overall methane yield (Angelidaki and Ahring, 1993). With organic solids such as solid manure residues, hydrolysis can also be the rate limiting step (Section 2.2.5). Optimum pH for hydrolysis is normally above pH 7.0, but hydrolysis is feasible down to pH 5.0, with lower pH risking inhibition (Babel et al., 2004; Dinamarca et al., 2003; Veeken et al., 2000; Zhang et al., 2005; Zverlov et al., 2010).

As leachbed processes operate with high solids content, inhibitor and/or toxicant concentrations can become elevated within the system and strongly affect digestion efficiency (Motte et al., 2013). Furthermore, recycling of solid digestate or leachate for inoculation could progressively accumulate inhibitors via carry-over. The manure component in solid manure residues also adds ammonia. The inhibitory effect of ammonia in AD has been well-documented (Rajagopa et al., 2013; Yenigun and Demirel; 2013). Previous studies have shown that methanogenesis is susceptible to total ammonia concentrations exceeding 1.5 g (as N) L^{-1} (Rajagopa et al., 2013; Yenigun and Demirel; 2013). Further, due to the downward shift of the ammonia acid-base pKa with increasing temperature, free ammonia concentration at a given total ammonia concentration increases with increasing temperature. This can contribute to process instability when treating wastes containing manure (Chen et al., 2008), and can be an issue with leachbeds starting up with a thermophilic phase (Section 2.4.2).

The digestion of solid manure residues could also be inhibited by humic substances (Fernandes et al., 2014). Humic acid (HA) is a product of the decay and/or polymerisation

of organic matter and behaves like a weak acid polyelectrolyte (Veeken and Hamelers, 1999). Previous studies have reported possible inhibition by humic substances within the concentration range 0.5 to 5.0 g L-1 (Fernandes et al., 2014; Ghasimi et al., 2016). Hydrolysis can be inhibited by HA (Brons et al., 1985; Fernandes et al., 2014) and mechanistic hypotheses have been proposed for this inhibition, including:

(a) threshold-type inhibition where HA binds to active sites of relevant hydrolytic enzymes, thereby preventing access to substrates (Brons et al., 1985), and/or

(b) that humic substances bind to hydrolytic bacterial cell walls, disrupting cell wall integrity and essential cellular processes, thereby affecting microbial activity (Smith et al., 2005).

In addition, Fernandes et al. (2014) indicated that humic compounds have a stronger affinity for enzymes that hydrolyse cellulose, as compared to enzymes that hydrolyse butyrin, suggesting that the inhibition may be selective towards particular enzymes and/or substrates. The mechanisms of HA inhibition is a subject of on-going research. Currently, the mitigation of HA inhibition is limited to physico-chemical means and there may be an opportunity to instead use inoculation with resilient microbial communities to overcome the chemical inhibition (see Section 2.5 below).

2.5 Summary of key knowledge gaps

Many have advocated for use of continuous digestion systems to treat solid wastes, because of performance reputation over batch systems. However, for the benefits stated above (Section 2.3), solid-phase batch systems are more likely to be feasible for solid manure residues in the decentralized on-farm context. The limitations of batch systems were clearly highlighted above (Section 2.3), in terms of relatively poor performance compared to continuous systems and the impact of inhibitors.

Process performance of leachbeds is partly dictated by leachate contact and mass transfer, with leachate movement, hold-up and/or channelling in the solid bed being key considerations (Section 2.4.1). Because mass transfer is so influential and intimately linked with leachate flow, leachate flow configuration can be very important for initial and on-going solid-liquid contact and performance of a leachbed. Previous studies have not compared different leachate flow configurations in parallel (flooded vs. trickling), which is important especially for solid manure residues, where characteristics of each batch of material to be treated can be substantially different (Section 2.1). Also, there is a need to test the leachbed at relevant scale to provide insight for future applications (Musolline, 2013).

Self-inoculation may be possible with leachbeds and solid manure residues, due to the presence of an indigenous microbial community on the residue (Section 2.4.2). This capability of self-inoculation is especially relevant for farm-scale AD due to limited availability of external inoculums in remote area and biosecurity restrictions which prohibits the flow of materials between farms. However, it remains unknown whether solid manure residue contain adequate microbial presence and species as well as the necessary ingredients, such as pH buffering agents to initiate AD (Section 2.4.3). Also, there has been limited study of the response of indigenous microbial community to different operating conditions, such as temperature, which can be particularly relevant with a thermophillic start-up, and high solids loading linked to mass transfer and inhibitor concentrations. Inoculation and digester start-up techniques (Section 2.4.2) are yet to be explored for leachbeds and solid manure residues, such as leachate sequencing or solid digestate recycling. These could be, potential means to speed up the start-up of a leachbed and to enhance the AD rate and performance.

Section 2.4.3 above highlighted the potential importance of HA inhibition with solid manure residues in leachbeds. In general, variations in AD reactor configurations, operating conditions and substrate compositions could be selectors for microbial composition and diversity within AD (McHugh et al., 2004; McHugh et al., 2003; Vanwonterghem et al., 2014), which could impact on inhibition resilience. For example, a review by Smith et al. (2005) suggested that bacteria could potentially overcome HA inhibition by producing altered enzymes and/or through membrane modification and repair. However, the review by Smith et al. (2005) only focused on humic-resistant bacterial strains found in the rumen, which may not be generally relevant to broader AD. Prior studies have focussed on physico-chemical methods to mitigate HA inhibition, such as precipitation or complexing with metal salts (Azman et al., 2015; Brons et al., 1985) or ion exchange (Boyer and Singer, 2006; Fearing et al., 2004). However, such methods are unlikely to be cost effective with AD of solid manure residues. It is of interest to identify links between AD performance and microbial activity and community composition, to determine whether inoculation with resilient communities could promote HA inhibition resilience and thus enhance AD.

The present thesis aimed to clarify the influence of the various factors noted above on leachbed digestion performance for solid manure residues. This is done to identify opportunities to optimize leachbed performance for this particular substrate of interest. Accordingly, a set of specific objectives were identified (Section 1.2) that are addressed by the thesis research.

CHAPTER 3
General materials and methods

This chapter describes general materials and methods shared by more than one chapter of the thesis.

3.1 Sampling

Spent bedding samples were collected from piggeries in Queensland as identified in separate chapters. The samples were typically collected from fresh stockpiles of bedding. Five samples were collected along the stockpile from a middle and deep layer of the pile (Figure 3.1). These samples were then combined and passed from a bucket to another bucket a number of times to mix thoroughly, before a composite sample was collected and prepared for further analysis as detailed in Sections 3.2 and 3.3 below. These samples were stored refrigerated at 4 °C before use (typically within a week).

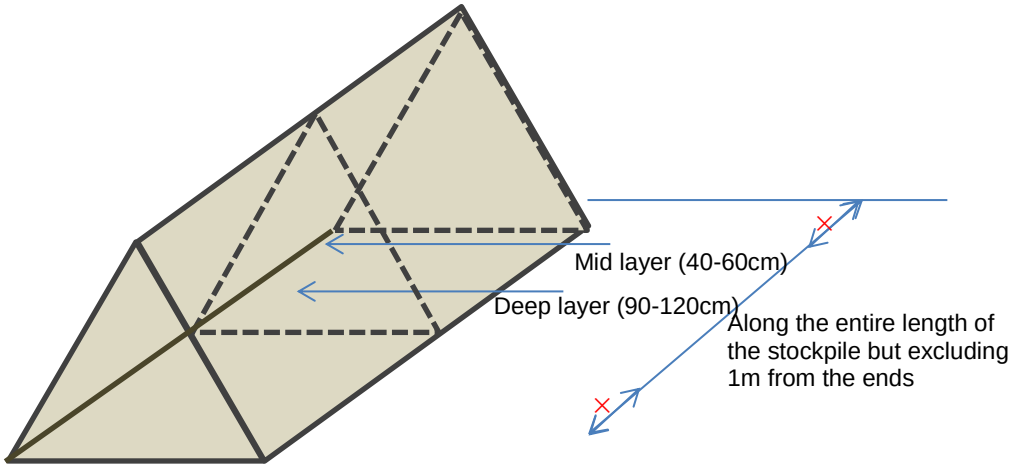

Figure 3.1. Indicative stockpile structure from where samples of solid manure residue were collected for testing, showing a mid and deep layer from where samples were collected.

3.2 Analysis of total fractions

For total fractions, smaller representative sub-samples were obtained from the composite sample above, by mixing the full composite thoroughly, and then taking a full vertical cross-section. The analysis was performed directly on these sub-samples, as described in Section 3.4 below.

3.3 Analysis of soluble fractions

For soluble fractions (dissolved constituents in water-liquid contained within a sub-sample), a methodology had to be developed to extract solubles from the sample, because such a method was not well-established. For this, a series of tests were conducted as described in Sections 3.3.1-3.3.3 below. These tests identified the preferred method to be diluting the sample with MilliQ® water at 1:10 weight ratio, and then mixing in a sample shaker for 30 minutes before filtering through 0.45 µm syringe filters (PES membrane) and storing the filtrate at 4 ºC until analysis. A single collected lot is dedicated for each test series to eliminate the influence of sample differences. The analysis was then performed on the extracts, as described in Section 3.4 below.

3.3.1 Method

Wheat-based spent beddings were used in this study. The validation method involved adding a weighed amount of residue sample to a measured amount of deionized water (either 1:5, 1:10 and 1:20 weight ratio). This mix was then either:

1) immediately filtered through a 0.45 µm syringe filters without additional mixing, blending or storing;
2) mixed in a sample shaker for 30 minutes before filtering like (1);
3) mixed in a sample shaker for 2 hours before filtering like (1);
4) left unmixed on the bench for 30 min before filtering like (1) (ambient room conditions of around 20 °C);
5) left unmixed on the bench for 2 hours before filtering like (1); or
6) left unmixed in the refrigerator for 2 days without mixing before filtering like (1).

3.3.2 Results

The filtrates were then analyzed for soluble Chemical Oxygen Demand (sCOD) and VFA concentration. The results were normalized to TS content for comparison (Table 3.1).

Table 3.1. Measured soluble properties of spent bedding of different extraction techniques.

Dilution (weight ratio)	Test condition (see above)	sCOD (g gTS^{-1})	VFA (g gTS^{-1})
1:5	1	0.06	0.0131
	2	0.10	0.0238
	3	0.13	0.0247
	4	0.14	0.0295
	5	0.14	0.0253
	6	0.09	0.0255
1:10	1	0.12	0.0354
	2	0.26	0.0469
	3	0.19	0.0455
	4	0.26	0.0382
	5	0.22	0.0396
	6	0.25	0.0409
1:20	1	0.12	0.0374
	2	0.27	0.0479
	3	0.21	0.0485
	4	0.27	0.0412
	5	0.26	0.0413
	6	0.26	0.0415

3.3.3 Conclusion

Based on the results, the optimal method was recommended: dilution at weight ratio of 1:10 and (2) mixed in a sample shaker for 30 minutes before filtering through a 0.45 µm syringe filters. This method gave the most reproducible results, with minimal chance of chemical and biological changes to occur during the sample preparation.

3.4 Chemical analysis

The analysis methods used for TS, VS, VFAs, total and soluble Chemical Oxygen Demand (tCOD and sCOD, respectively), nitrogen species such as total ammonium–nitrogen (TAN), and phosphate-phosphorus, were the same throughout the thesis, and are thus described below. For total fractions, the analysis was performed directly on samples as collected. For

soluble fractions, the analysis was performed on the filtrate stored at 4°C (Section 3.3). Where measured data are presented throughout the thesis with error bars or error (±), these are average values (n ≥ 3) with error representing a 95% confidence interval based on a two-tailed *t*-test (5 % significance threshold). Where relevant, errors were analytically propagated as described by Batstone (2013).

3.4.1 TS and VS

These analyses were performed according to Standard Methods 2540 (Rice, 2012), in triplicate. Samples, of approximately 10 g for inoculums or 30 g for spent bedding, were added to a clean crucible (pre-weighted as W_c) and the total weight of sludge and empty crucible were recorded as W_i. The crucibles were first dried in an oven (105 ˚C) overnight (minimum of 12 h) and weighed the next day after cooling to room temperature in a desiccator over silica gel for the calculation of TS (W_1) (Equation 3.1). The crucibles were then heated in a 550 ˚C furnace for 3 h, cooled to room temperature in a desiccator and reweighed (W_2). The VS was taken to be the difference between W_1 and W_2 (Equation 3.2).

$$TS\% = \frac{W_1 - W_c}{W_i - W_c} \times 100 \quad (3.1)$$

$$VS\% = \frac{W_1 - W_2}{W_i - W_c} \times 100 \quad (3.2)$$

Where W_c = weight of empty crucibles (g)
W_i = initial weight of material and empty crucible (g)
W_1 = weight of dried residue and crucible (g)
W_2 = weight of material and crucible after furnace (g)

The typical analytical uncertainty in the analysis of VS and TS was within ± 5 %.

3.4.2 tCOD and sCOD

Chemical oxygen demand for the soluble fraction (sCOD) was determined using Merck Spectroquant® COD cell tests (#14541 and #14555). Samples were quantitatively diluted where necessary to be within the detection range. All COD measurements were performed in replicates of at least three for filtered samples, at least three for slurries and at least five

for solid samples. Photometric measurements were performed using a Spectroquant® Move 100 mobile colorimeter (Merck, Germany), with a MilliQ water blank subtracted as a background. The typical analytical uncertainty in the analysis of sCOD and tCOD was within ± 10 %.

3.4.3 VFA

VFA concentrations were measured using a Perkin Elmer gas chromatograph (GC) with a free fatty acid phase (FFAP) capillary column (Agilent Technologies, USA). High purity helium was used as carrier gas at a flow rate of 17 mL min^{-1}. The injection port, oven and detector were operated at 220, 140 and 250 ˚C, respectively. For this analysis, pre-filtered samples (0.45 µm PES) were diluted 1:10 (weight ratio) with MilliQ® water. 0.9 mL of diluted samples were then preserved by adding 0.1 mL of 10 % formic acid and storing at 4 ºC until analysis. The typical analytical uncertainty in the analysis of VFA was within ±5 %, respectively.

3.4.4 Nitrogen species and phosphate

TAN, nitrate (NO_3^-), nitrite (NO_2^-) and phosphate-phosphorus (PO_4^{3-}) were measured on pre-filtered samples (0.45 µm PES) using a Lachat QuickChem800 Flow Injection Analyser (FIA) (Lachat Instrument, Milwaukee). Filtered samples were typically diluted 1:50 (weight ratio) with MilliQ water to make up a total volume of 5 mL. The analytical method was based on the Berthelot reaction (Rice, 2012). The typical analytical uncertainty in the analysis of nitrogen and phosphate species was within 5 %, respectively.

3.4.5 Dissolved Organic Matter and Humic substances

For the Chapter 4 and 5 experiments, the concentration of humic substances was determined using a liquid chromatography- organic carbon and nitrogen detector (LC-OCD-OND) Model 8 according to the method of Huber et al. (2011) at the Water Research Centre of the University of New South Wales. Leachate sample was filtered through 0.45 µm and diluted 1:100 (weight ratio) with MilliQ water to make up to a 50 mL sample volume before storing in 50mL polypropylene tube sent to the University of New South Wales for analysis. For Chapter 5 experiments, dissolved organic matter (DOM) was characterised via excitation emission matrix (EEM) fluorescence by a PerkinElmer LS-55 luminescence spectrometer (PerkinElmer, Australia) (Chen et al., 2003).

3.5 Biochemical methane potential (BMP) tests

Biochemical methane potential (BMP) tests were performed to estimate anaerobic biodegradability and the rate of anaerobic digestion under substrate-limited conditions, ie. at a high inoculum-substrate ratio (ISR) on a VS basis. These results were then used to establish baseline biochemical methane potential (B_0) for comparison with methane yields observed under test conditions in Chapters 4 and 5.

BMP tests followed the methods of Angelidaki et al. (2009). Batch digestion was conducted in 310 mL non-stirred glass serum bottles (250 mL working volume). Inoculum for the BMPs was collected from a mixed mesophilic sewage digester in South East Queensland (Australia) treating primary and secondary municipal sludge. The inoculum was typically added at an ISR of 2 on a VS basis. Test bottles were flushed with 100% N_2 gas for about 1 min (4 L min^{-1}), after which they were immediately sealed with a rubber septum retained with an open top screw cap. The test bottles were then stored in an incubator at 37 ± 1 °C. Biogas samples were periodically collected from the headspace of each bottle for measurement of methane produced. Biogas volume was measured using a manometer and methane content was determined by GC as described in Section 3.5. The test bottles were mixed by swirling before and after every sampling event, but not in-between sampling events. Tests were performed in triplicate and background methane production from blanks (substrate-free assay) were subtracted. The batch experiments were terminated when the net methane produced over three consecutive days was less than 1 % of the cumulative methane produced up to the last of those three days (Holliger et al., 2016)

The BMP test data were analysed using the software package Aquasim 2.1d (Jensen et al., 2011). This analysis performed a non-linear least-squares fit of a simple first-order plus dead time kinetic model (Equation 3.3) to measured data of cumulative methane (B_t) produced over incubation time (t) (Jensen et al., 2011):

$$B_t = B_0(1 - e^{-k.t}) \quad (3.3)$$

where k is the first-order degradation rate coefficient, and B_0 and k have units of mL CH_4 g VS_{fed}^{-1} and d^{-1}, respectively.

The degradation extent (f_d) was then estimated using the value of B_0 as follows:

$$f_d = \frac{B_0 \times gVS_{fed}}{380 \times gCOD_{fed}} \times 100 \quad (3.4)$$

with the value of 380 mL CH_4 g COD^{-1} being the theoretical conversion of methane to g COD at 25 ºC and 101.3 kPa. A 95 % confidence interval in both parameters f_d and B_0, were estimated based on a two-tailed *t*-test (5 % significance threshold) with standard error determined using a Secant Fisher information matrix as per Jensen *et al.,* (2011). Where relevant, errors were analytically propagated as described by Batstone (2013).

3.6 Biogas analysis

Biogas composition (CH_4, CO_2 and H_2) was determined using a Shimadzu GC-2014 GC equipped with a Valco GC valve (1 mL sample loop), a HAYESEP Q 80/100 packed column (2.4 m length; 1/8 inch outside diameter, 2 mm inner diameter) and a thermal conductivity detector (TCD). The chromatograph injector, oven and detector temperatures were set at 75, 45 and 100 °C, respectively. Argon was used as carrier gas at a flowrate of 28 mL min^{-1} and pressure of 135.7 kPa. The GC was operational 24/7 and was calibrated every 6 months (because background calibration did not drift significantly over time) with 3 different calibration gases comprised of various CH_4, CO_2 and H_2 concentrations. The relative uncertainty in the biogas analysis was typically within ± 5 %.

3.7 Microbial community profiling

Microbial community profiling was used in Chapters 5 and 6. Genomic DNA was extracted by using a FastSpin for Soil Kit (MP-Biomedicals, Santa Ana, California, USA) according to the manufacturer's protocol. 300 ng DNA of each sample were provided to the Australian Centre for Ecogenomics (The University of Queensland) for 16S Amplicon sequencing using a Illumina Miseq Platform with a 926F (5'-AAACTYAAAKGAATTGACGG-3') and 1392wR (5'-ACGGGCGGTGWGTRC-3') primer set (Engelbrektson et al., 2010). Raw paired reads were first trimmed by Trimmomatic (Bolger et al., 2014) to remove short reads (less than 190bp) and low quality reads (lower than Phred-33 of 20). The trimmed paired reads were then assembled using Pandaseq (Masella et al., 2012) with default parameters. The adapter sequences were removed by FASTQ Clipper of the FASTX-Toolkit (Pearson et al., 1997).

The joined high quality sequence was analysed using QIIME v1.8.0 (Caporaso et al., 2010) with an open-reference operational taxonomic units (OTU) selecting strategy by uclust (Edgar, 2010) at 1 % phylogenetic distance and assigned taxonomy by uclust against the greengenes database (13_05 release, McDonald et al. (2012); Werner et al. (2012)). OTUs with only one read were filtered from the OTUs table by command filter_otus_from_otu_table.py in QIIME. Filtered OTUs table were imported to Galaxy (Giardine et al., 2005) for gene copy number correction and generate final absolute abundance of each OTU by CopyRighter (Angly et al., 2014)

CHAPTER 4
Leachbed pilot trials

Process performance of leachbeds is partly dictated by leachate contact and mass transfer, with leachate movement, hold-up and/or channelling in the solid bed being key considerations (Section 2.4.1). Previous studies have investigated different modes of leachate flow (flooding and trickling), with the aim to enhance mass transfer and thus leachbed performance (Section 2.4.1). However, there have not been parallel comparisons of different leachate flow configurations. This is important for solid manure residues to determine a preferred leachate flow mode to improve leachbed performance (Section 2.4.1). Such a study is presented in this chapter, where leachbed digestion of spent bedding from pigs/swine is conducted at the relevant pilot-scale of 200 L. The effect of external inoculation is also compared with AD exclusively via self-inoculation by the spent bedding substrate.

4.1 Materials and methods

4.1.1 Materials

Spent bedding samples were collected from stockpiles at two piggeries at Nanango and Goondiwindi (Site A and B, respectively) in Queensland (Australia) and analysed as described in Chapter 3. The samples were essentially “fresh” from a pig shed, with stockpiles being 0 - 2 days old at the time of sampling. At both sites, the spent bedding was from sheds housing smaller pigs (weaners). The pigs were all reared according to a batch “all in, all out” mode (Section 2.1), but the batch time of Site A and B was different at 6 and 4 weeks, respectively. This meant that spent bedding from Site A was expectedly more soiled than spent bedding from Site B (Section 2.1). Moreover, bedding at Site A consisted only of wheat straw, whilst bedding at Site B contained 40 % wheat straw, 40 % barley straw and 20 % sorghum straw (on a weight basis of fresh bedding added to the pig sheds).

4.1.2 Pilot-scale equipment

A pilot scale leachbed system was setup in single stage mode (Section 2.3). The design of the system underwent several changes to resolve issues with clogging, as highlighted below. Early preliminary trials and leachbed development of the present work are also described in Appendix A.

Leachbed reactors

Two leach bed reactors were setup, differing in operating method as trickling and flood-and-drain illustrated in Figure 4.1 and Figure 4.2, respectively. Each leachbed had a total working volume of 200 litres, which permitted loading with a representative 15 kg quantity of the typically heterogeneous spent bedding. The leachbed reactors were constructed from 316 stainless steel (SS) to prevent corrosion. For each leachbed, a SS perforated plate was installed near the base of the vessels on which the bed of solid waste rested while being contacted with leachate.

In the trickling leachbed, the mesh base plate was made from 304 SS, with a net open area of 41 % and 3 mm openings (Figure 4.3). A spiral spray nozzle was installed below the lid of the leachbed reactor to evenly distribute leachate over the top of the bed of solid waste.

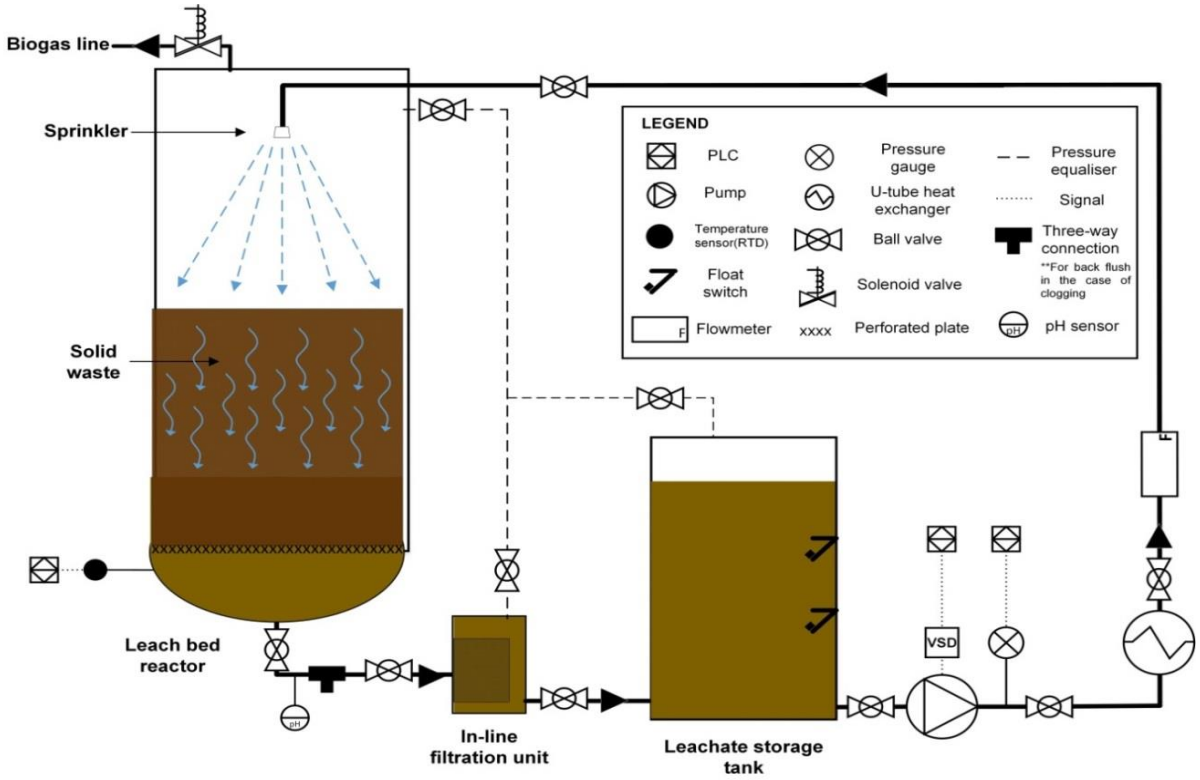

Figure 4.1. Trickling single-stage leachbed reactor with sprinkling system

In the flood-and-drain leachbed, the perforated base plate was made from 304 SS mesh with 5 mm openings (Figure 4.3). The perforation size of the base plate in this leachbed was later changed to 3 mm, because of continuous issues with clogging (Section 4.1.3 below). To minimize the liquid dead-volume in the flood-and-drain leachbed, the mesh base-plate was close to the bottom of the flood-and-drain leachbed. Multiple triangular conduits made of 304 SS mesh with 5mm openings were positioned vertically, parallel to each other, within the solid waste bed of the flood-and-drain leachbed. These conduits provided channels for effective distribution and drainage of leachate during flooding and draining, respectively.

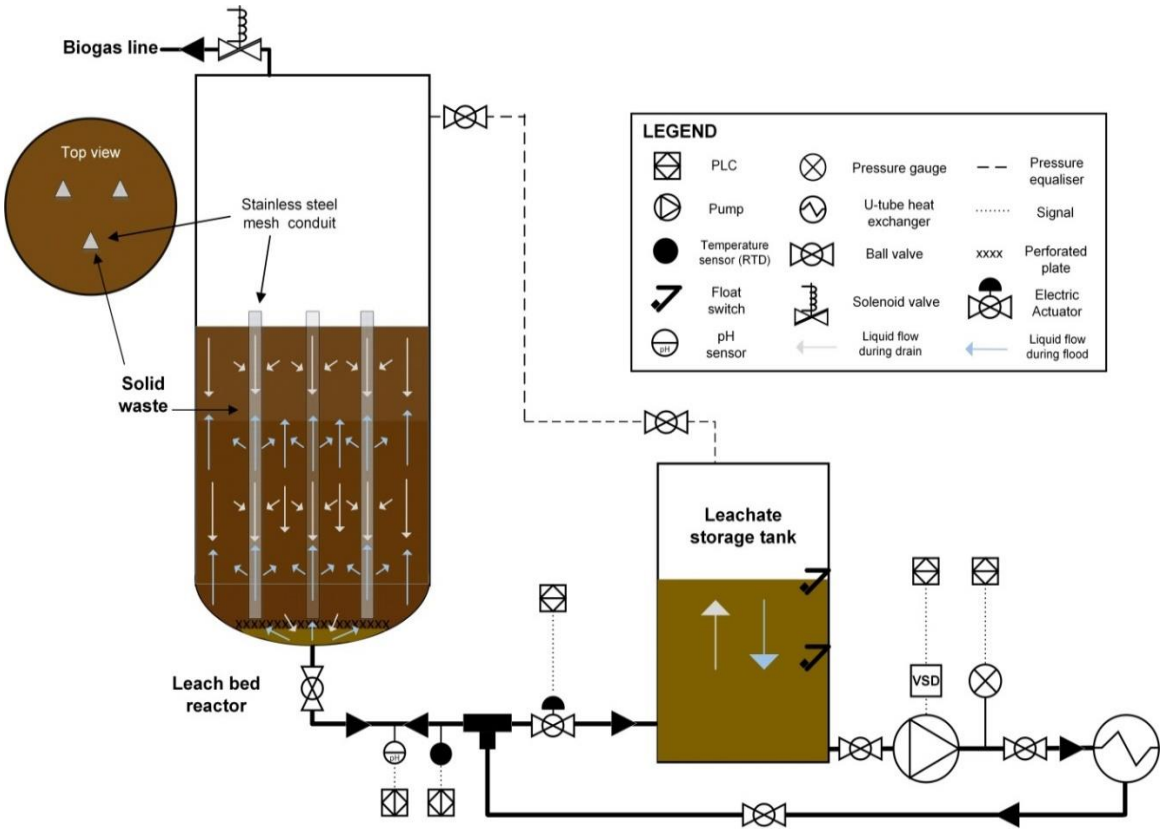

Figure 4.2. Flood-and-drain single-stage leachbed with conduits vertically imbedded in the solid waste bed to allow an even distribution of leachate.

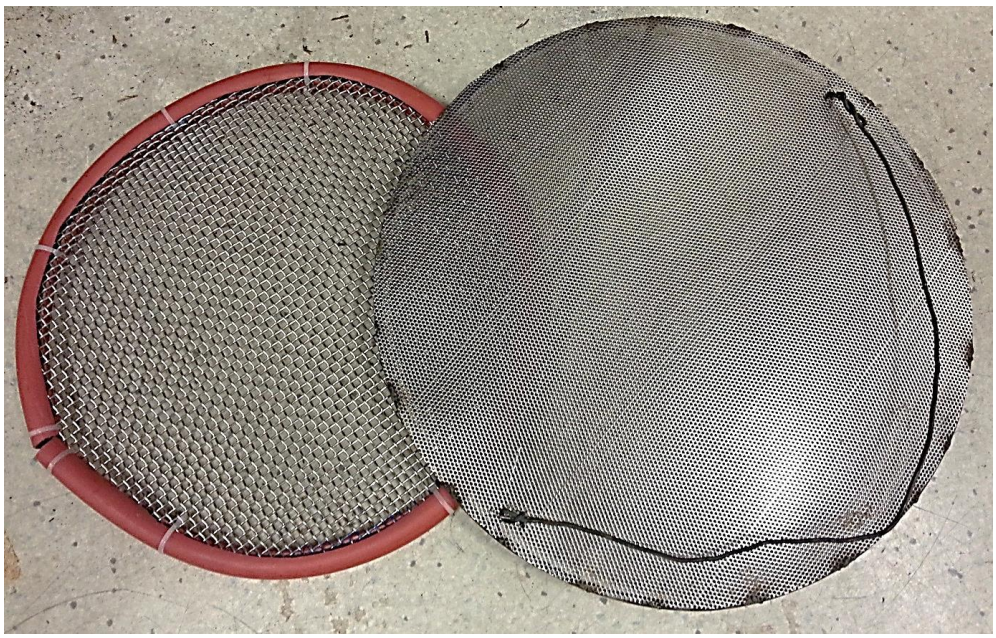

Figure 4.3. Perforated plate for (left) the flood and drain system (5 mm openings) and (right) the trickling system (3 mm openings) used in Test 1 (Section 4.1.3).

The outer surfaces of the leachbed vessels were covered with 50 mm graphite impregnated foam insulation, to help retain heat and maintain leachbed temperature. Biogas was released via a gas outlet on the lid of the leachbed, connected to a SS cooling coil that condensed moisture to flow back into the leachbed. A SS solenoid valve in the biogas line closed in a timed manner at the same time that leachate was added/removed. This was done to prevent large fluctuations in headspace gas pressure and thus allow accurate measurements of biogas volume being produced.

Leachate recirculation

Each leachbed had an adjacent 120 L high density polyethylene drum that served as a leachate storage tank to collect leachate from the leachbed and allow recirculation. The leachate storage tank was air tight to maintain anaerobic conditions and to allow further collection of biogas. The biogas pipeline of the leachate storage tank was connected to the main biogas pipeline from the leachbed.

In the flood-and-drain system, leachate was recirculated back to the leachbed reactor to fully flood the solid waste bed for 6 hours, before the leachate was drained back out into the leachate storage tank by gravity through an opened drain valve.

In the trickling system, the recirculated leachate percolated by gravity through the solid waste bed in the leachbed reactor and flowed back into the leachate storage tank via an in-line strainer. This in-line strainer was installed after preliminary leachbed trials (Appendix A) had experienced issues with coarse particulate or fibrous bedding clogging the spray nozzle (see Figure 4.4).

The leachate recirculation pump was controlled by means of liquid level float switches installed in the leachate drum. Leachate recirculation was initiated when the high liquid level float switch was triggered. Leachate was then pumped back into the leachbed reactor by a progressive cavity pump (CP11, Monopump) at 2 L min^{-1}. Once the low liquid level float switch was triggered, the recirculation pump switched off. Then, once the liquid high level float switch was again triggered by leachate flowing from the leachbed back into the leachate storage tank, the leachate recirculation sequence was again initiated. The timing of liquid level rise from the low level switch up to the high level switch allowed a measure of any changes in hydraulic conductivity across the bed of solid waste (Appendix A), due to for instance compaction (Section 2.4.1). An inline pressure transducer (UNIK 5000, GE) also protected the pump, by switching the pump off, if the pressure downstream of the pump was to increase to excessive levels (e.g. if the leachate recirculation line became blocked).

Figure 4.4. (Left) Spiral cone sprinkler clogged by straw in preliminary leachbed trials without an inline strainer; (Middle) Straw pieces that caused the blockage. The blockage was subsequently resolved by installing an inline strainer (Right).

Temperature control

The temperature of each leachbed was maintained by heating the leachate as it flowed via a heat exchanger. The heat exchanger (Figure 4.5), akin to a shell-and-tube configuration, consisted of a 110 L cooler as the “shell” being heated by a thermoregulator (TH6P, Ratek), and 18 of half-inch SS tubes with a combined heat transfer area of about 0.6 m^2. The thermoregulator temperature was set at 40 ˚C. The temperature of the leachbed reactor was measured as an operating condition, using a resistance temperature detector (RTD) sensor (model SEM203 P, WandB Instrument Pty). Operating temperature of leachbed reactor was typically observed to be 37 ± 2 ºC.

Figure 4.5. Heat exchanger used to heat leachate in order to maintain a mesophilic temperature in each leachbed.

Biogas measurement

Volumetric biogas production from each leachbed and its adjacent leachate storage drum was measured using a positive displacement manometer, consisting of a U-tube filled with silicone oil (50 centistoke, Dow Corning), a relay float switch, a timer, a mechanical counter

and a solenoid valve. The float switch tripped when there was sufficient build-up of headspace pressure and opened the solenoid valve to allow the biogas to vent to a vent-line. The gas meter was calibrated by measuring the volume of gas required to trip the float switch. The biogas production was then determined from the number of counts multiplied by the calibrated volume per count. A biogas sample was routinely collected from a modified Hungate-type anaerobic test tube with butyl rubber stopper, using a gas-tight syringe and fine-gauge needle. This tube was located downstream of the SS cooling coil described above and upstream of the manometer. Biogas composition was analysed as described in Section 3.6.

4.1.3 Test conditions

Two separate leachbed trials (termed Test 1 and 2) were carried out, each with the two leachbeds operating in parallel. The difference was that in Test 1 the leachbeds were started without adding an external inoculum, simply relying on native microbial activity of the manure in the spent bedding, whereas in Test 2 the leachbeds were inoculated with solid residue and leachate from the trickling leachbed of Test 1. Each trial ran for about 50 days (dictated by the levelling off of biogas production over time). Table 4.1 summarises the initial set-up conditions of Tests 1 and 2.

Table 4.1: Initial start-up conditions of leachbeds in Test 1 and 2.

	Test 1	Test 2
Spent bedding origin	Site A	Site B
Fresh substrate load (kg, wet basis)	15	10
Solid digestate as inoculum (kg, wet basis)	n/a	5
ISR (VS basis)	n/a	0.22
Liquid fraction		
Water (kg)	97.5	40
Leachate (kg)	n/a	40
Initial system TS (% weight ratio, wet basis)	6.2 ± 0.2	5.7 ± 0.2

In Test 1, the mesh sizes of the supporting base plate used in the trickling and the flood-and-drain leachbeds were 3 mm and 5 mm, respectively. The larger mesh size in the flood-and-drain leachbed was to facilitate free-flow of leachate during the flood and drain cycles. However, midway through Test 1 (day 25), the leachate recirculation line of the flood-and-

drain leachbed became continuously clogged by fibrous material that passed through the mesh base plate when leachate was being drained out. This issue was resolved in Test 2 by changing the aperture size of the baseplate to instead be 3 mm, to better retain fibrous material in the flood-and-drain leachbed.

During the course of Tests 1 and 2, 10 mL leachate samples were intermittently collected from the leachate storage tank to analyse for TS, VS, VFA, tCOD, sCOD, TAN, NO_3^-, NO_2^-, PO_4^{3-} and humic substances (Section 3.4). At the same time, samples of headspace gas were collected from an inline gas sample point for composition analysis by GC (Section 3.6). At the end of each trial, leachate was drained from the remaining solid residue in each leachbed over a 24 h period, before the remaining solid residues and collected leachates were separately further analysed for TS, VS, VFA, tCOD, sCOD, TAN, NO_3^-, NO_2^-, PO_4^{3-} and humic substances, as described in Section 3.4. Free ammonia nitrogen concentration (g NH_3-N L^{-1}) was then calculated for inhibition analysis, using Equation 4.1 (Batstone et al., 2002):

$$S_{NH3} = \frac{K_{a,NH4} \cdot S_{IN}}{K_{a,NH4} + 10^{-pH}} \quad (4.1)$$

The acid-base equilibrium coefficient for ammonium ($K_{a,NH4}=10^{-9.25}$) was corrected for leachbed temperature T (degrees K) using the constant enthalpy form of the van't Hoff relation with a heat of reaction of 51.965 kJ $Mole^{-1}$ (Batstone et al., 2002).

BMP tests were conducted as described in Section 3.5, to provide baseline methane yields for the spent bedding substrates with which methane recovery from each leachbed could be compared.

4.1.4 Residual methane potential tests

Residual methane potential was measured for the remaining solid residue and leachate collected at the end of each leachbed trial. These measurements used the BMP methods described in Section 3.5, but with and without added inoculum. The residual methane potential was quantified as the measured cumulative methane normalised to the amount of VS present in the residue being tested. In the case without added inoculum, the solid residues were diluted with milliQ water at a dilution factor of 0, 2 or 4 or with leachate at a dilution factor of 2 or 4. These additional tests were performed to assess suspected effects of chemical inhibitors and mass transfer limitations.

4.1.5 Data analysis

Substrate solubilisation was quantified as the sum of COD contributions by methane produced (COD_{CH4}) plus soluble COD measured in leachate samples ($sCOD_{leachate}$), normalized to the tCOD originally added ("fed") to the leachbed (Equation 4.2):

$$\text{Extent of substrate solubilisation } (\%) = \left(\frac{\text{COD}_{\text{CH}_4} + \text{sCOD}_{\text{leachate}}}{\text{tCOD}_{\text{fed}}}\right) \times 100 \quad (4.2)$$

Methane productivity was quantified as a ratio of methane COD and tCOD originally fed to the leachbed (Equation 4.3):

$$\text{Extent of methane conversion}(\%) = \left(\frac{\text{COD}_{\text{CH}_4}}{\text{tCOD}_{\text{fed}}}\right) \times 100 \quad (4.3)$$

These methods were adapted from Jensen et al. (2011). Note that tCOD originally fed to the leachbed in Test 2, also included the COD contribution of added inoculum (leachate and solid digestate). The extents of substrate solubilisation and methane production were calculated over time for each sampling event.

As a check of data quality, a COD balance was performed at the end of each leachbed trial using Equation 4.4:

$$\text{COD mass balance } (\%) = \left(\frac{\text{COD}_{\text{CH}_4} + \text{tCOD left over in residual solid and leachate}}{\text{tCOD}_{\text{fed}}}\right) \times 100 \quad (4.4)$$

to confirm that tCOD initially fed into the leachbed was accounted for by COD contributions of methane recovered during the leachbed trial plus COD remaining in the solid residue and leachate at the end of the trial. A secondary quality check was also performed on methane yields using Equation 4.5, comparing the methane potential (as calculated from B_0) of the spent bedding originally added to the leachbed, with the summed contributions of cumulative methane recovered from the leachbed during the trial (COD_{CH4}) plus residual methane potentials of the solid residue and leachate at the end of the trial. Residual methane potential was separately measured by the BMP method described in Section 3.5

$$\text{Methane balance } (\%) = \left(\frac{\text{BMP of recovered leachate and residual solids } + \text{COD}_{\text{CH}_4}}{\text{BMP of original substrate fed to the leachbed}}\right) \times 100 \quad (4.5)$$

4.2 Results and discussion

4.2.1 Materials characteristics

Table 4.2 summarises key characteristics of the raw spent bedding from Sites A and B. VS/TS ratios and k measured by BMP testing were similar for bedding from Sites A and B. B_0 was only slightly lower for bedding from Site A than for bedding from Site B. Measured B_0 values were comparable with those reported elsewhere (Kusch et al., 2008; Tait et al., 2009; Tong et al., 1990). The nutrient content of the spent beddings was comparable to those reported by Craddock and Wallis (2013).

Table 4.2. Measured spent bedding characteristics from deep litter housing at Site A and B in Queensland, Australia.

Parameter	Site A	Site B
TS (%)	45 ± 2	42 ± 3
VS (%)	35 ± 2	31 ± 2
VS/TS ratios	0.77 ± 0.02	0.73 ± 0.03
TAN (g NH_4-N kg^{-1} TS dry basis)	4.3 ± 0.2	4.7 ± 0.2
Phosphate-phosphorus (g PO_4-P kg^{-1} TS dry basis)	0.57 ± 0.02	1.69 ± 0.10
VFAs (gVFAs kg^{-1} TS dry basis)	2.6 ± 0.2	3.0 ± 0.2
B_0 (L CH_4 kg^{-1} VS_{fed})	195 ± 8	218 ± 9
k (d^{-1})	0.15 ± 0.01	0.14 ± 0.01

4.2.2 Pilot-scale leachbed performance

Figure 4.6 and Figure 4.7 present time series results for the leachbed trials with and without inoculation, respectively. Overall, the COD balance closed exceptionally well within 90 ± 9 %. Further, 94 ± 8 % of the B_0 was accounted for by methane produced plus residual methane potential in the solid digestate and leachate. These observations indicated that the measured data quality was high and that any methane losses during the trials were negligible.

In Test 1 up to day 25 (Figure 4.6 a, b), the trickling system and flood-and-drain systems had comparable performance, but at day 25 the leachate recirculation pipelines in the flood-

and-drain leachbed became clogged, as noted earlier in Section 4.1.3. Once leachate could no longer be recirculated through the heat exchanger in the flood-and-drain leachbed because of this clogging, the measured operating temperature in this leachbed rapidly decreased from 37 ˚C down to room temperature (25 ˚C). As expected, this decrease in operating temperature disrupted methanogenic activity (Batstone and Jensen, 2011) and biogas production ceased (Figure 4.6 b). Propionate rapidly accumulated, but stopped soon after, possibly due to product inhibition (Veeken and Hamelers, 1999). Due to these issues, the discussion below on Test 1 results considers the full dataset for the trickling leachbed in Test 1, but only considers data for the flood-and-drain leachbed collected up to day 25. All the data for both leachbeds of Test 2 were deemed valid and were used in the discussion and interpretation.

Initially in Test 1, soluble organic matter (measured as sCOD) rapidly accumulated in both leachbeds (Figure 4.6 a, b), possibly due to rapid mobilisation of water-soluble organic matter from the raw spent bedding. Subsequently, sCOD gradually declined and stabilised after 40 days. VFA accumulation was detected shortly after start-up, with acetic acid being initially prominent, before declining as propionic acid accumulated (Figure 4.6 c, d). In the trickling system, all VFAs were consumed to exhaustion within 40 days with no net production observed thereafter (Figure 4.6 c, d). VFA accumulation decreased the pH of the leachate to below 6.0, but pH recovered as VFAs were converted into methane (Figure 4.6 e, f). Methane production appeared to be initially delayed by low pH, but gradually recovered (Figure 4.6 a, b). Methane production in the trickling system levelled off at around 40 days, with an overall extent of substrate solubilisation (CH_4 + sCOD) of 44 % and a methane conversion of 51 % of the B_0 (Figure 4.6 a, b) (the B_0 was 195 L CH_4 kg^{-1} VS_{fed}). Methane concentration measured in the headspace gas was considered to be reasonable at up to 58 ± 3 % by volume.

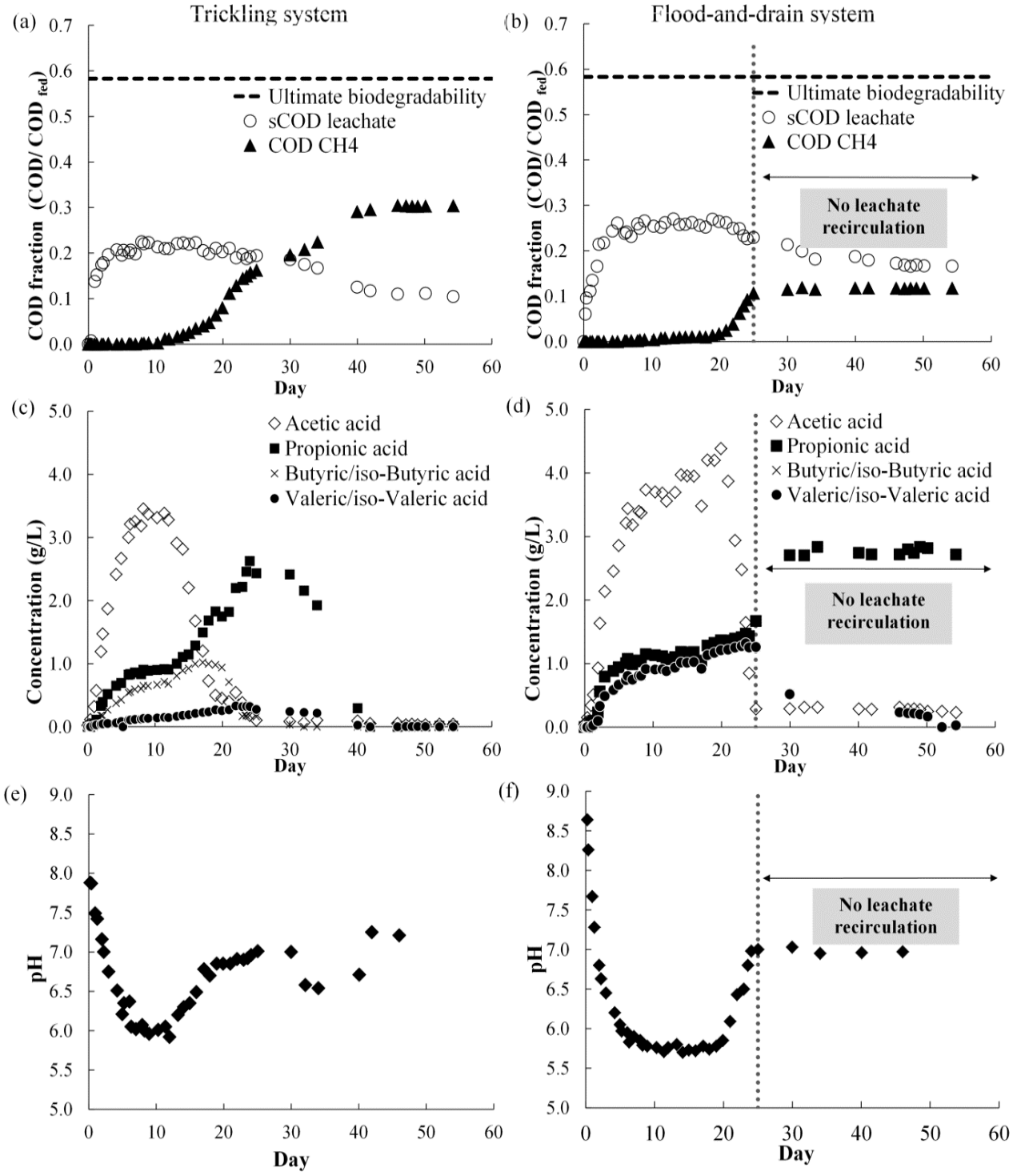

Figure 4.6. Leachbeds performance from Test 1, showing time trends of sCOD in leachate ($sCOD_{leachate}$) and cumulative methane produced in COD units (COD_{CH4}) (a - b), VFAs (c - d), and pH (e - f). Data on the left is for the Trickling leachbed, and data on the right is for the flood-and-drain leachbed. The horizontal dashed lines in (a) and (b) is the biodegradability of the spent bedding as measured in the BMP testing.

The two leachbeds in Test 2 achieved comparable methane recovery, although the extent of substrate solubilisation was slightly higher in the flood-and-drain system than in the trickling system. VFAs rapidly accumulated at start-up but stabilised after day 16 with no net production observed after that. Acetic and propionic acid were the most notable VFAs (Figure 4.7 c, d). Unlike in Test 1, VFA accumulation in Test 2 caused a comparatively minor decrease in pH (Figure 4.7 e, f), likely due to effects of the added inoculum (alkalinity and microbial activity). Likely for the same reason, methane production in Test 2 commenced near instantaneously (Figure 4.7 a, b). Methane production in Test 2 was initially steady but ceased after day 16 (Figure 4.7 a, b).

Overall, both the leachbeds in Test 2 achieved an extent of substrate solubilisation (CH_4 + sCOD) of around 30 % and a methane recovery of about 50 % of the expected B_0 (the B_0 was 194 L CH_4 kg^{-1} VS_{fed}, including the inoculum contribution). This indicated poor leachbed performance ad further discussed below. Comparing the results from Test 1 with the results from Test 2, the Trickling leachbed in Test 2 (with added inoculum) achieved the same methane conversion within half the digestion time taken by the Trickling leachbed in Test 1 (without added inoculum). A shorter digestion time is generally desirable from a leachbed size and cost perspective, so inoculation strategies are further explored in Chapters 5 and 6.

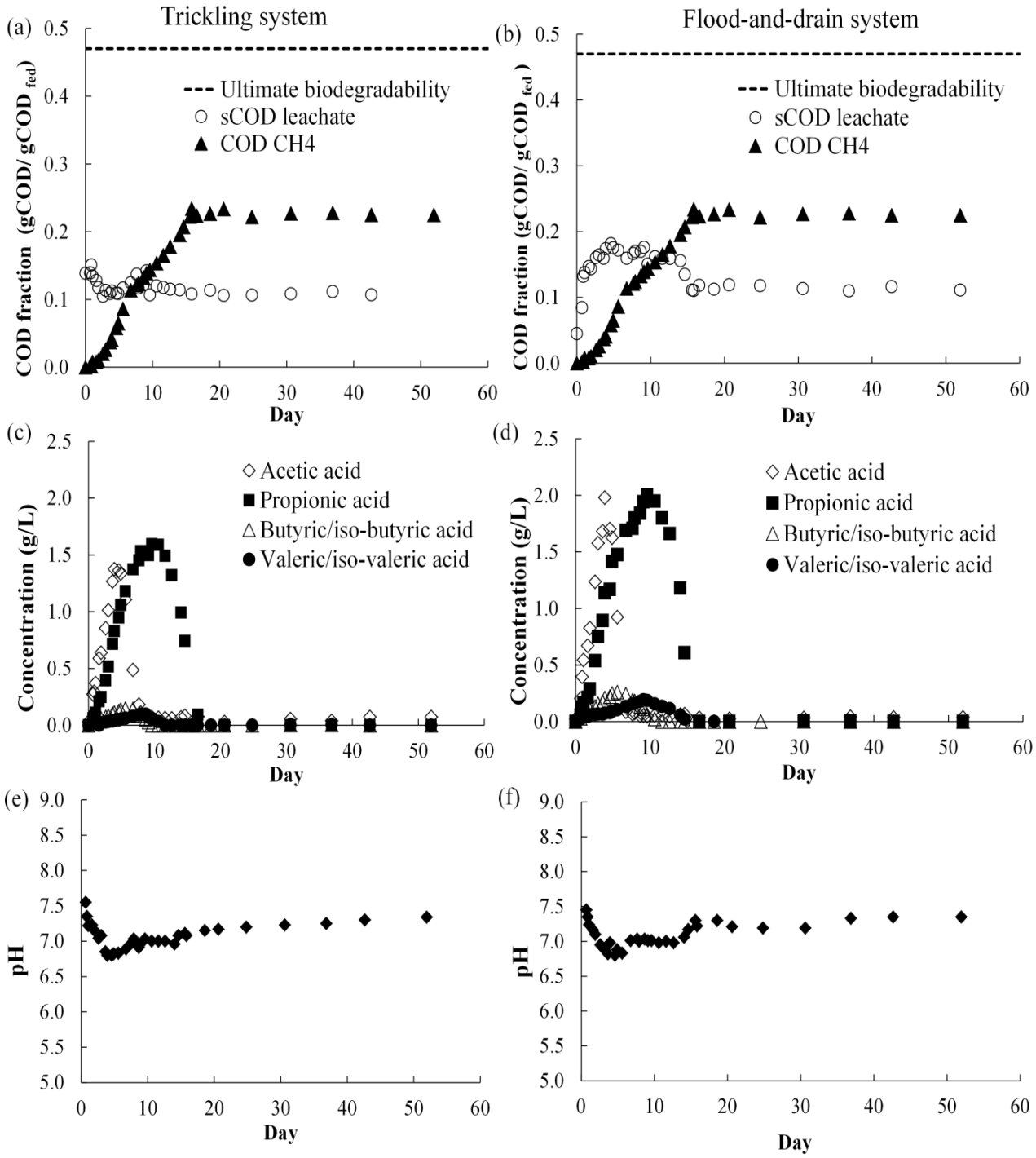

Figure 4.7. Leachbed performance from Test 2, showing time trends of sCOD in leachate ($sCOD_{leachate}$) and cumulative methane produced in COD units (COD_{CH4}) (a - b), VFAs (c - d), and pH (e - f). Data on the left is for the Trickling leachbed, and data on the right is for the flood-and-drain leachbed. The horizontal dashed lines in (a) and (b) is the biodegradability of the spent bedding as measured in the BMP testing.

4.2.3 Digestate analysis

Table 4.3 presents characteristics of the residues from Test 2.

Table 4.3. Measured characteristics of residues collected at the end of Test 2.

Parameter	Trickling	Flood-and-drain
Solid residue		
TS (%)	20 ± 2	13 ± 3
VS (%)	1 5 ± 2	9.7 ± 3
Ammonia (mg NH_4-N g TS^{-1})	2.54 ± 0.05	3.55 ± 0.06
Phosphate- phosphorus (mg PO_4-P gTS^{-1})	0.34 ± 0.02	0.42 ± 0.03
Leachate		
TAN (mg NH_4-N L^{-1})	604 ± 44	639 ± 42
Soluble phosphate-phosphorus (mg PO_4-P L^{-1})	66 ± 6	52 ± 3
Humic substance concentration (g L^{-1})	1.2 ± 0.2	1.6 ± 0.2

Figure 4.8 shows results of the fractionation of $tCOD_{fed}$ as methane, leachate and solid digestate for each leachbed at the end of Test 2. This data indicated that the flood-and-drain system mobilised more COD into the leachate than the trickling system (Figure 4.9 a). However, the COD mobilised by the flood-and-drain leachbed contained a large fraction of non-biodegradable material (Figure 4.9 b), which is unwanted because of little potential for post-processing into methane and because such entrained particulates can cause materials handling issues at full-scale. The SS conduits used in the flood-and-drain leachbed (Section 4.1.2) could have increased the entrainment of solids. Previous studies have shown that lignocellulosic waste can act as a viable filter media to promote separation of particulate solid from the liquid phase (Zhang and Lorimor, 2000). Thus, the absence of lignocellulosic materials in the conduits likely facilitated the migration of particulates with leachate. A flood-and-drain leachbed without conduits may experience less mobilisation of suspended solids.

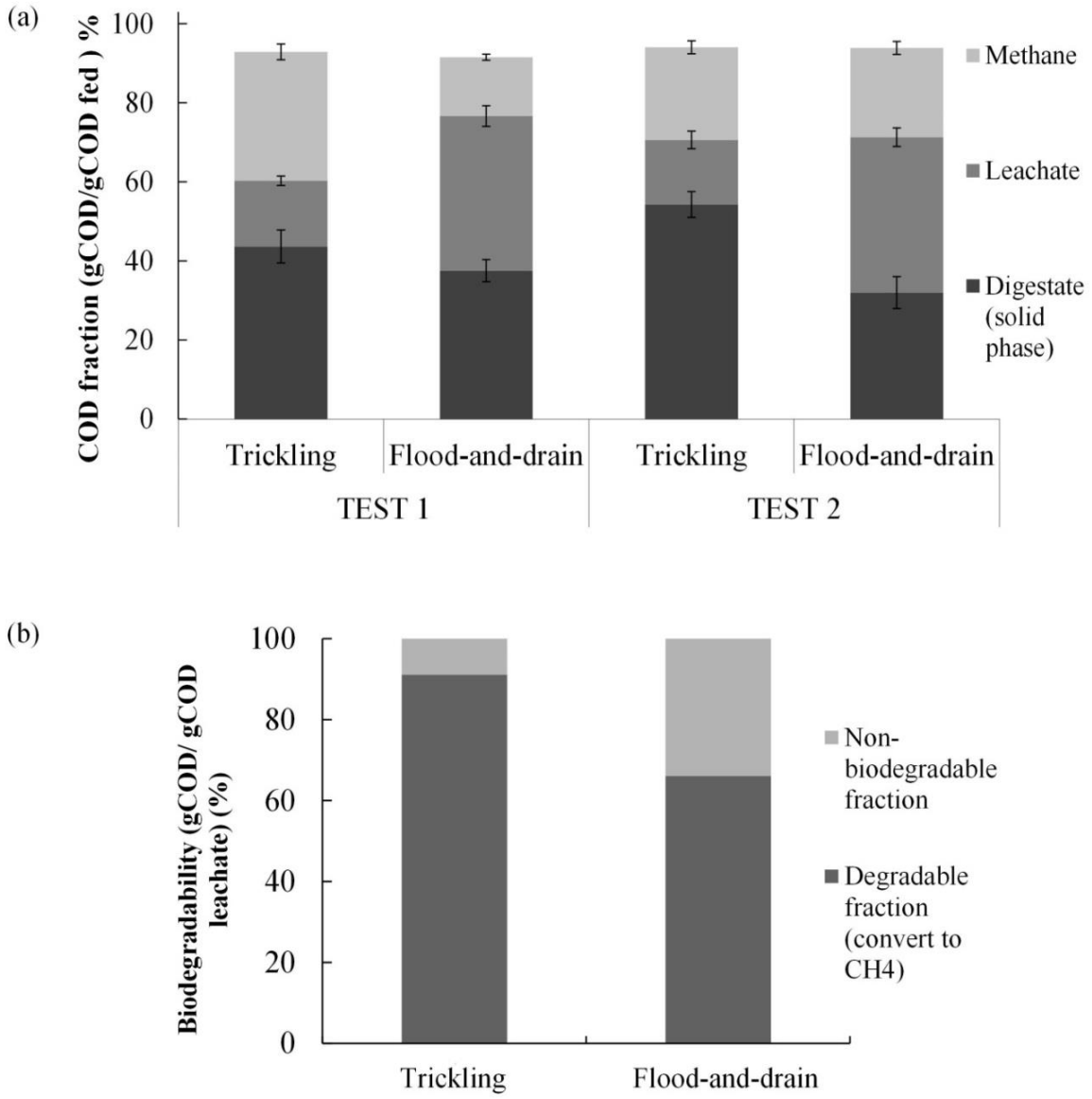

Figure 4.8. Residue analysis: (a) Methane, leachate and solid residues in g COD as percentage of g COD_{fed} for the pilot-scale trials after 50 days of operation; (b) Post-digestion residual anaerobic biodegradability analysis of leachate from Test 2.

Moisture recovery from leachbed at the end of each experiment was 96 - 98 % of the initial process water input, with the remaining process moisture likely retained in the saturated solid residues (Aikaterini, K., 2015) or lost via the biogas line.

The residual methane potential was high for the leachate recovered at the end of Test 2 (732 ± 10 L CH_4 kg^{-1} VS_{fed} for trickling and 582 ± 8 L CH_4 kg^{-1} VS_{fed} for flood-and-drain), indicating poor leachbed performance but good potential for post-processing of leachate into methane (Section 4.2.4). These residual methane potentials corresponded to about 25 and 30 % of the B_0 of the spent bedding, respectively.

4.2.4 Post-processing for methane recovery

Figure 4.9 presents results from tests of post-processing for additional methane recovery with and without an added external inoculum (water or leachate dilution only). These tests were performed on solid residue collected from the leachbeds at the end of Test 2. Based on the results, the full residual B_0 could only be recovered when an external inoculum was provided; indicating that indigenous microbial activity in the spent bedding was insufficient. However, in the absence of external inoculum, the solid residues did continue to degrade into methane (Figure 4.9), even though methane production had ceased in the leachbeds of origin (Figure 4.6 and 4.7). A higher methane productivity was observed when the solid residues were more diluted with water or leachate, suggesting an impact of mass transfer limitations in the original leachbeds. Such mass transfer limitations could result due to effects of leachate channelling through the solid bed. The composition of the leachate and solid residues are expected to be complex and a range of inhibitors could be present (Section 2.4.3). Inhibition testing (Astals et al., 2015) was performed on samples of the solid residue from the leachbeds. These results suggested that background ammonia in the leachbed was not at inhibitory levels (Figure 4.10). Whilst most inhibitory compounds may be at low concentrations, some compounds can also be inhibitory at very low levels (Chen et al., 2008). The effects of chemical inhibition and microbial activity are further explored in Chapter 5 (inoculation) and Chapter 6 (inhibition).

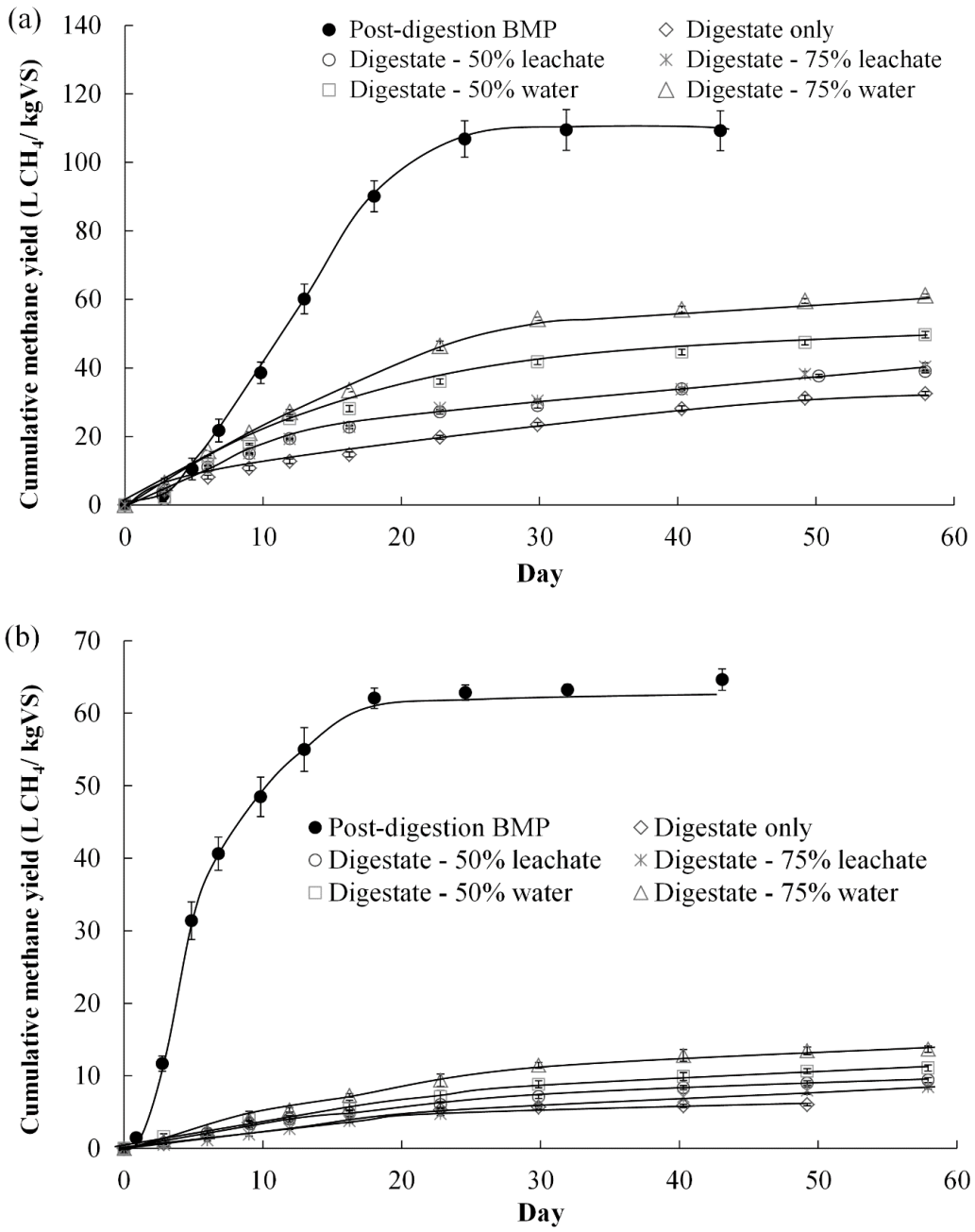

Figure 4.9. Post-digestion residual methane analysis of solid residue diluted with leachate and water at different dilution factors to assess the mass transfer limitation and biological inhibition of leachbeds in Test 2: (a) trickling; (b) flood-and-drain.

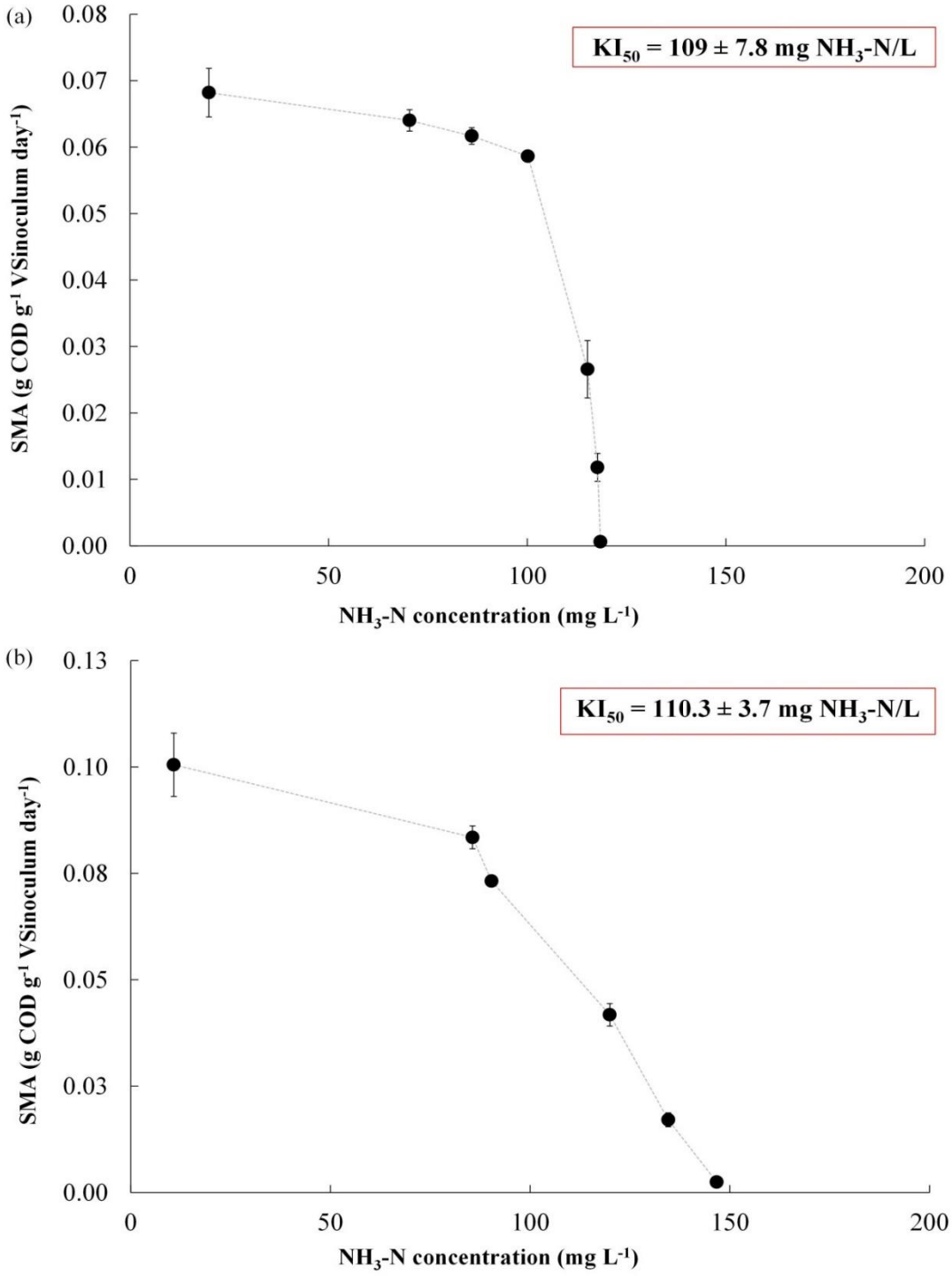

Figure 4.10. Inhibition testing for ammonia using solid residues from Test 2 leachbeds as inoculum: (a) trickling; (b) flood-and-drain. Inhibition coefficient (KI_{50}) indicated biostatic inhibition potential of ammonia, representing the concentration at which the uptake rate is half the maximum.

4.2.5 Implications for future farm-scale applications

Process performance in leachbeds is heavily influenced by leachate contact and mass transfer, both of which are likely to be influenced by the mode of leachate flow. The results indicated that a trickling arrangement is preferred over a flood-and-drain arrangement. Specifically, both the trickling and flood-and-drain type leachbed achieved comparable methane conversion (50 % of the B_0), despite their markedly different modes of leachate flow (Section 4.2.2). However, the flood-and-drain leachbed mobilized more unwanted non-biodegradable particulates (Section 4.2.3), which could complicate leachate handling and likely require more maintenance.

The target system at full-scale could be on-farm decentralized with a leachbed operating in tandem with an existing covered anaerobic pond. Post-processing for additional methane recovery was shown to be possible (Section 4.2.4) and leverages existing infrastructure. In this way, methane recovery can occur from both the leachbed and the covered anaerobic pond, and the treated outflow from the covered pond might be recycled to the leachbed as leachate (Figure 4.11). Future research should further explore the performance impacts of using treated covered lagoon effluent as a leachate.

Given that covered anaerobic lagoons are now increasingly being accepted in Australian agriculture (The Department of Agriculture, Fisheries and Forestry, 2008), the addition of a leachbed is considered to be a conceivable incremental change to onsite practices. Multiple leachbeds could operate in parallel single-stage to provide continuous biogas production. Based on the leachbed trial results (section 4.2.2), the majority of methane recovery occurs within 20 days of leachbed operation. At pig farms, this aligns well with typical all-in-all-out systems, where a large batch of spent bedding is produced when a finished batch of pigs leaves a shed. For example, each leachbed could operate for about 28 days split into: 21 digestion days (solids retention time) and 7 additional days for substrate loading/unloading, cleaning and maintenance. As an example, the single-stage pilot-scale leachbeds recovered 50 % of the B_0 (100 L CH_4 kg^{-1} VS_{fed}) for spent bedding from pigs/swine. A 1000 pig deep litter system produces about 0.8 dry tonne of VS per day (Tait et al, 2009). This would amount to approximately 1440 GJ of energy. The skill level requirements for operation would be manageable for typical on-farm applications.

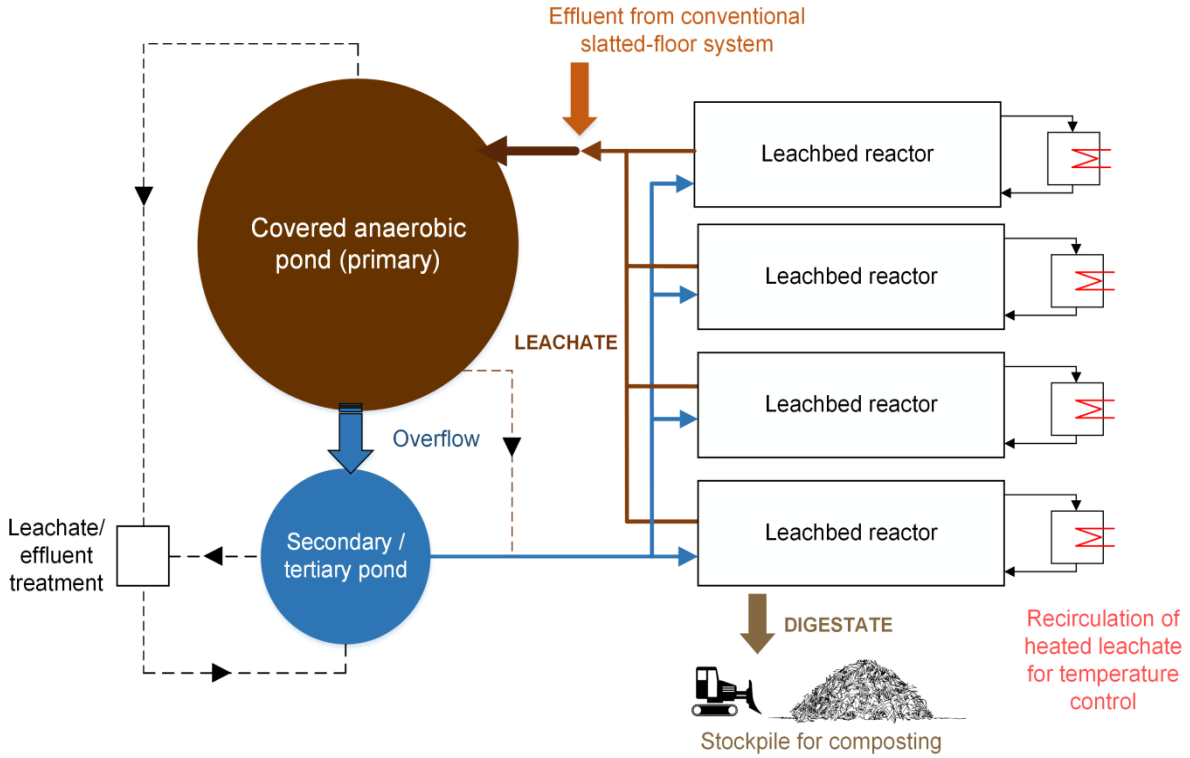

Figure 4.11. Schematic depiction of a possible leachbed setup at farm-scale. This setup considers the use of existing infrastructure to reduce baseline capital cost, and also keeps the operational complexity to a minimum to facilitate adoption.

From the findings of the post-processing analyses (section 4.2.4), it was not clear what caused the poor 50% methane recovery of the leachbed, whether performance was limited by inadequate inoculation or whether performance was rather impacted on by chemical inhibitors. These potential limitations of leachbed performance were thus further explored in Chapter 5 (inoculation) and Chapter 6 (inhibition), with the aim of understanding key limitations and accordingly enhance leachbed performance.

CHAPTER 5

The effect of operating conditions on solid-phase digestion of solid manure residues

This chapter evaluates the effects of solids concentration, temperature and inoculation on AD of spent bedding. Solids concentration (5 - 20% TS) is investigated with a view on solid-phase digestion such as in a leachbed (Chapter 4). The experiments utilize indigenous microbial community in the spent bedding for self-inoculation, and the results are compared with separate tests using digestate and leachate residue from a previous digestion batch as a different means of on-going inoculation. This is done to better understand inoculation options, also under different start-up scenarios of temperature and solids concentration. The outcomes future-proof applications by clarifying the operating conditions/factors that limit the performance of leachbeds.

5.1. Materials and Methods

5.1.1 Materials

Spent bedding was collected and prepared for further analysis as described in Chapter 3. “Fresh” bedding samples (0-2 days old at the time of sampling) were collected from stockpiles at two piggeries in Nanango and Goondiwindi (Site C and D, respectively) Queensland (Australia). The spent bedding from Site C was from sheds housing smaller pigs only (weaners, 10 - 24 kg), whereas spent bedding from Site D was from sheds housing weaners and larger pigs (growers, 24 - 36 kg). At both sites, the pigs were all reared according to a batch “all in, all out” mode (Section 2.1). The batch time of the weaner-to-grower was 4 weeks at Site C and 6 weeks at Site D. At Site D, the time for grower pigs to grow to slaughter weight was 3 weeks. It was clear from visual observation that these batch times translated into different extents of exposure of the spent bedding to pigs, because the bedding from Site C contained less faeces and urine (less soiled) than bedding from Site D. The average bedding use at Sites C and D were about 0.30 and 0.12 kg pig^{-1} day^{-1}, respectively. Bedding at Site C consisted of mixed barley straw and wheat straw (50 %, weight ratio, each) and was collected for testing during winter, when in-shed temperature was an estimated 20 ± 5 ˚C. Bedding at Site D consisted of wheat straw only and was collected during summer, when in-shed temperature was an estimated 27 ± 8 ˚C. The bedding samples were analysed for TS, VS, VFA, tCOD, sCOD, TAN, NO_3^-, NO_2^- and PO_4

$^{3-}$ in accordance with the methods described in Section 3.4. BMP tests were also conducted according to the method described in section 3.5 for a baseline methane potential with which to compare methane yields under test conditions in this chapter.

5.1.2 Batch tests without inoculation

Batch experiments were conducted at a similar scale and using a similar method as for the BMPs, except that no external inoculum was added. This was to assess the impact of the indigenous microbial activity in the spent bedding on AD performance. The test conditions, as outlined in Table 5.1, included combinations of solids concentrations of 5, 10 and 20% TS, achieved by diluting the spent bedding with MilliQ water, and test temperatures of 37 or 55 ˚C .

Table 5.1. Test conditions of the batch experiments without external inoculum.

Spent bedding sample origin	Temperature (T, ˚C)	TS (%)	Test analysis performed
Site C	35	5	Gas composition and soluble content
		10	
		20	Gas composition
	55	5	Gas composition and soluble content
		10	
		20	Gas composition
Site D	35	5	Gas composition and soluble content
		10	
		20	Gas composition
	55	5	Gas composition and soluble content
		10	
		20	Gas composition

Tests at each condition were performed in sextuplicate to compensate for spent bedding heterogeneity. During the course of a test set, 1 mL leachate samples were intermittently collected from three of the six bottles via an 18 Gauge syringe needle and soluble fractions were analysed for sCOD and VFA in accordance with the method in Section 3.4. The exception was that liquid samples could not be collected from batch tests at 20 % TS,

because of inadequate free-draining liquid in these test bottles. Biogas production and biogas composition were analysed for all six bottles by the method in Section 3.6 and was corrected for changes in gas headspace volume by liquid sampling for the relevant three bottles at each test condition. The residue (leachate residual and solid digestate) from each test bottle was subsequently analysed for TS, VS, VFA, tCOD, sCOD, TAN, NO_3^-, NO_2^- , PO_4^{3-} and humic substances by the methods described in section 3.4.

5.1.3 Batch tests with inoculation

To evaluate the activity of solid digestate and leachate from a prior batch as an inoculum for a subsequent batch, a separate batch test set was performed in accordance with conditions outlined in Table 5.2. This test was conducted at a fixed TS of 10 % and a test temperature of 37 ˚C. Tests inoculated with solid digestate were diluted with MilliQ water to 10 % TS, whereas tests inoculated with leachate were diluted with full-strength or diluted leachate (50 % with MilliQ water) to 10 % TS. The chosen solid digestate to fresh spent bedding mix ratio was assumed to be sufficient for spent bedding digestion, based on a study by Kusch et al. (2008).

Table 5.2 Test conditions for batch tests with inoculation.

Spent bedding origin	Inoculum source	Inoculation method	ISR (VS basis)
Site C	From previous batch tests at 5 % TS and 35 ˚C that had no external inoculum added, and that digested spent bedding from Site C	20 % solid digestate and 80 % fresh spent bedding on a weight basis	0.07 ± 0.01
		Full-strength leachate only	0.08 ± 0.01
		Diluted leachate (50% with MilliQ® water) only	0.04 ± 0.01
Site D	Batch tests at 5 % TS and 35 ˚C that had no external inoculation added, and that digested spent bedding from Site D	20 % solid digestate and 80 % fresh spent bedding on a weight basis	0.12 ± 0.1
		Full-strength leachate only	0.06 ± 0.01
		Diluted leachate (50 % with MilliQ® water) only	0.03 ± 0.01

* VS content of full strength leachate from Site C and D is 1.05 and 0.93 % (wet basis), respectively

The microbial community composition of the solid inoculum was analysed according to the method in Section 3.7. Background methane production contributed by the leachate and solid digestate added as inoculum was separately measured and subtracted. However, the amount of residual methane produced from the added inoculum was generally small relative to the amount of methane produced from the spent bedding substrate.

5.1.4 Data analysis

To analyse the methane production data from batch tests in Sections 5.1.3 and 5.1.4, a two-step model was setup in Aquasim 2.1d (Jensen et al., 2011) for AD process rate and yield parameter identification. The effect of TS levels was not explicitly incorporated in the model, because the complex process interactions between mass transfer and biological inhibition was considered to be beyond the scope of the study. Also, instead, the effect of TS was empirically captured by the comparison of estimated parameters for various TS concentrations. In the two-step model, particulate substrate (X_S, g COD g COD_{fed}^{-1}) was degraded to soluble monomers (S, g sCOD g COD_{fed}^{-1}) at a rate r_x (g COD g COD_{fed}^{-1} d^{-1}) and subsequently converted into methane (g COD_{CH4} g COD_{fed}^{-1}) at a rate r_s (g COD g COD_{fed}^{-1} d^{-1}) as in Equations 5.1-5.3:

$$X \xrightarrow{r_x} S \xrightarrow{r_s} methane \tag{5.1}$$

$$r_x = -k_{hyd} * X_S \tag{5.2}$$

$$r_s = -\begin{cases} 0\,, \ and\ t \le t_d \\ k_{meth} * S, \ and t \ge t_d \end{cases} \tag{5.3}$$

where k_j is the first-order kinetic rate coefficient for process j (d^{-1}) denoted as "hyd" and "meth" for the biological processes of hydrolysis and methanogenesis, respectively, and t_d (units of d) is a lag-phase for methanogenesis during start-up. The lag phase for solubilisation (t_d, a fitted parameter) was generally negligible (i.e., not significantly or quantitatively different from zero), but was significant as measured for methanogenic activity. The initial condition for biodegradable particulates ($X_{S,0}$) was set to the total particulate substrate (X_{tot}) multiplied by the biodegradable fraction ($X_{S,0} = X_{tot} \times f_d$), where f_d is substrate biodegradability. Initial conditions for other state variables were zero. With batch conditions for which soluble fractions could not be measured (See Section 5.1.2 above),

methane production data (cumulative methane COD) was instead fitted with a simple first-order with lag phase kinetic model, to determine kinetic rate and degradability parameters.

5.2. Results and discussion

5.2.1 Material characteristics

Table 5.3 summarizes key characteristics of the spent bedding samples from Site C and D. Mobile nutrients, such as TAN and phosphate, as well as VFA concentration were higher in the spent bedding from Site D than from Site C, which could reflect a greater extent of soilage and/or pre-fermentation in the pig sheds of Site D (Section 2.1). This was not surprising, given the longer pig batch time and lower bedding quantity used per pig in Site D than at Site C (Section 5.1.1). Further, a higher in-shed temperature at Site D than at Site C (Section 5.1.1) could have promoted bacterial fermentation, thus resulting in higher mobilisation of nutrients and production of VFAs. B_0 values and anaerobic biodegradability were higher for the spent bedding from Site D. Specifically, the B_0 values measured for spent bedding from Site C was 140 L CH_4 kg VS_{fed}^{-1}, and for Site D it was 227 L CH_4 kg VS_{fed}^{-1}. These B_0 values were comparable to values reported elsewhere by Kusch et al. (2008) and Tait et al. (2009).

Table 5.3 Characterization results for spent bedding samples.

Spent bedding origin	Site C	Site D
TS (%)	39 ± 3	31 ± 2
VS (%)	27 ± 2	23 ± 2
VS/TS ratios	0.70 ± 0.01	0.74 ± 0.01
tCOD (kg COD kg TS^{-1} dry basis)	0.67 ± 0.04	0.86 ± 0.07
TAN (g NH_4-N kg TS^{-1} dry basis)	2.1 ± 0.2	4.6 ± 0.6
Phosphate-phosphorus (g PO_4-P kg TS^{-1} dry basis)	2.1 ± 0.3	1.4 ± 0.2
VFA (g VFA kg TS^{-1} dry basis)	2.3 ± 0.4	3.7 ± 0.9
B_0 (L CH_4 kg VS_{fed}^{-1})	140 ± 6	227 ± 7
f_d (%)	44 ± 4	58 ± 5
k (d^{-1})	0.12 ± 0.01	0.10 ± 0.01

Figure 5.1 shows microbial community composition of the spent bedding samples from Site C (left) and Site D (right). With the spent bedding from Site C, only 2 to 3 % of the total DNA extracted was identified as archaea, and similarly with the spent bedding from Site D, 4 to 6 % of total DNA extracted was identified as archaea (Figure 5.1). These results are further discussed below in Section 5.2.3, in terms of potential influence on AD start-up and performance. In both spent bedding samples, the identifiable archaea were dominated by *Methanobacterium* and *Methanosphaera*, with a lesser contribution by *Methanoculleus, Methanosarcina* and *Methanosaeta*. The bacteria community of both spent beddings was dominated by the phyla Bacteroidetes, Actinobacteria and Proteobacteria (Figure 5.1). Of these, Bacteriodetes and Proteobacteria, are known to be hydrolytic bacteria groups and degrade lignocellulosic material (Samet et al., 2014).

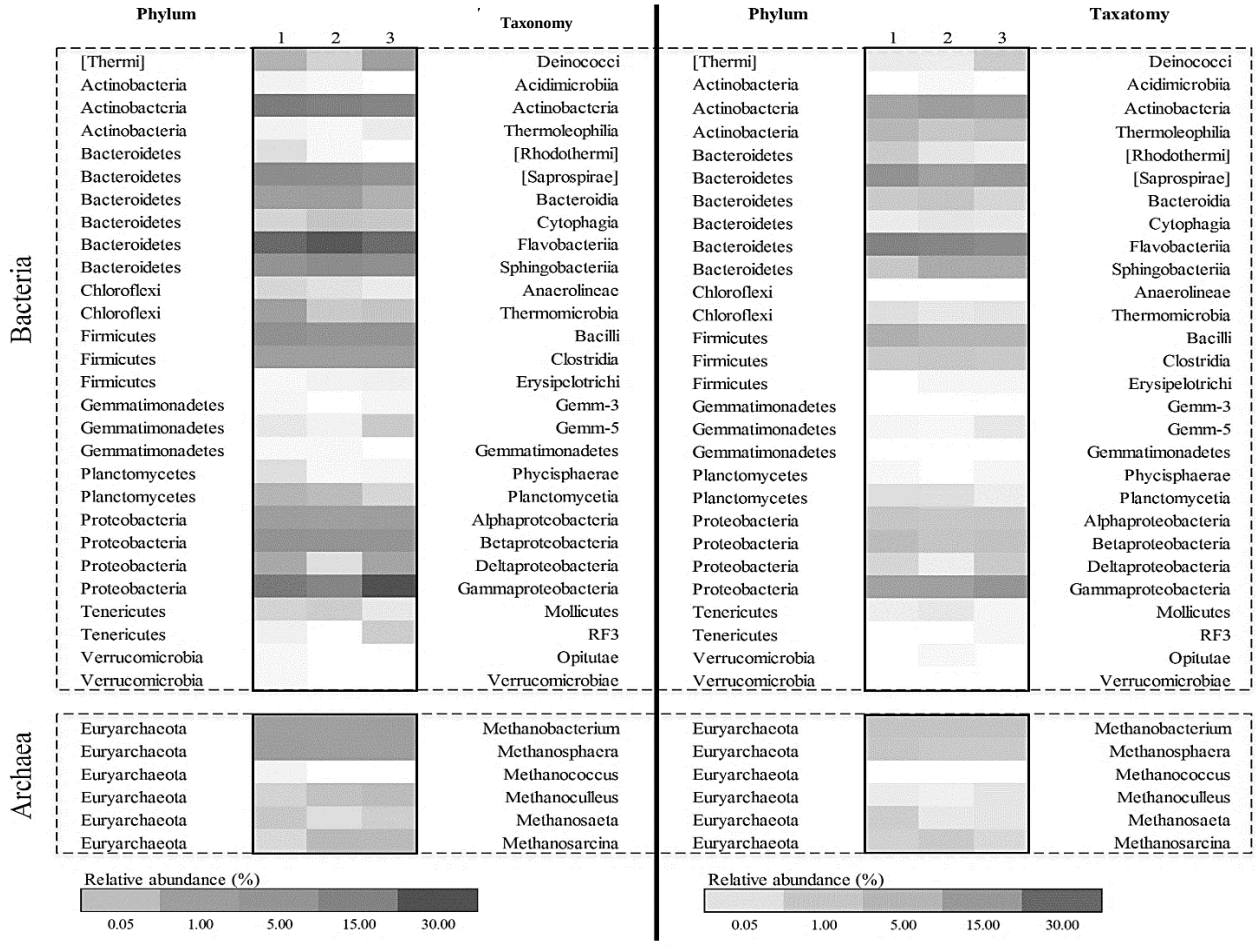

Figure 5.1. Microbial community composition of fresh spent bedding from Site C (left) and D (right) (> 1 % relative abundance). The heat map shows the relative abundance of microbial groups present on fresh spent bedding in triplicate (1, 2 and 3). The taxonomic classification is shown at the phylum level (left-hand side) and class and genus level of taxonomic assignment for bacteria and archaea, respectively, at 97% similarity.

5.2.2 Effect of solids concentration and temperature

Figures 5.2, 5.3 and 5.4 present time series data for the batch experiments without an external inoculum (Section 5.1.2) and Table 5.4 below summarises digestion parameters obtained from kinetic model fits of the data. The results suggested that hydrolysis followed first-order kinetics without a time lag, whereas methane production followed first-order kinetics with a time lag. Hydrolysis rate decreased with increasing TS for both spent bedding samples, as indicated by a lower value for k_{hyd} at higher TS (Table 5.4). At each respective TS, hydrolysis was marginally faster at 55 ˚C than at 35 ˚C, as indicated by slightly higher k_{hyd} values at 55 ˚C.

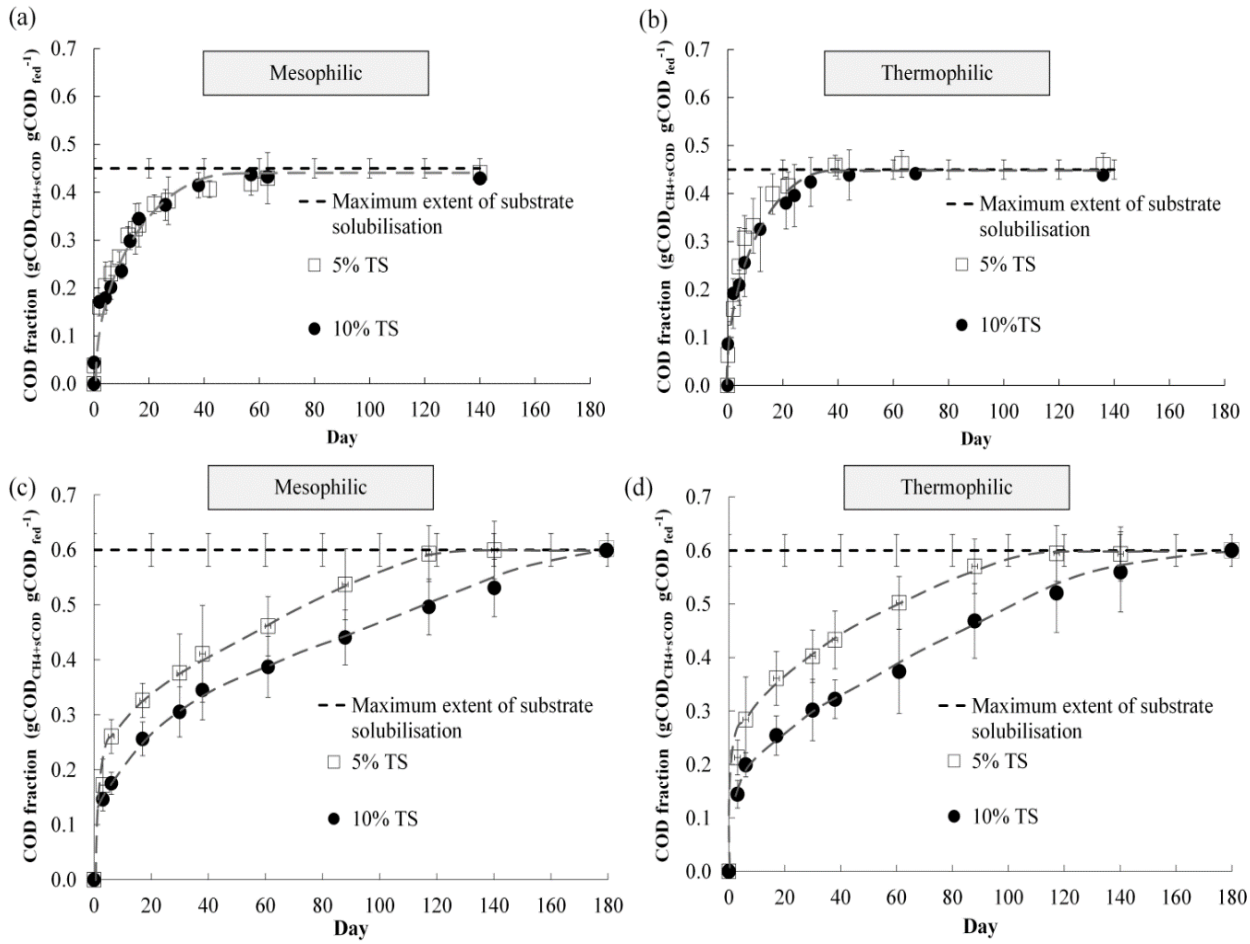

Figure 5.2. The hydrolysis extent of spent bedding from Site C ((a); (b)) and D ((c); (d)) during batch experiment without external inoculation.

Substantial VFA accumulation occurred at start-up (4 - 16 g VFA L^{-1}), but was consumed over time as methane was being produced (Figure 5.3 and 5.4). As expected, the extent of VFA accumulation increased with increasing TS and was also slightly higher at 55˚C than at 35˚C (Figure 5.3). For both bedding samples, total VFA at 10 % TS was nearly double that at 5% TS, most likely due to the higher organic loading at the higher TS. The VFA profiles were comparable between the two temperatures, with the exception of a higher peak VFA at 55 ˚C than at 35 ˚C. Acetic acid was dominant, followed by propionic acid contributing a lesser but still major proportion of total VFAs.

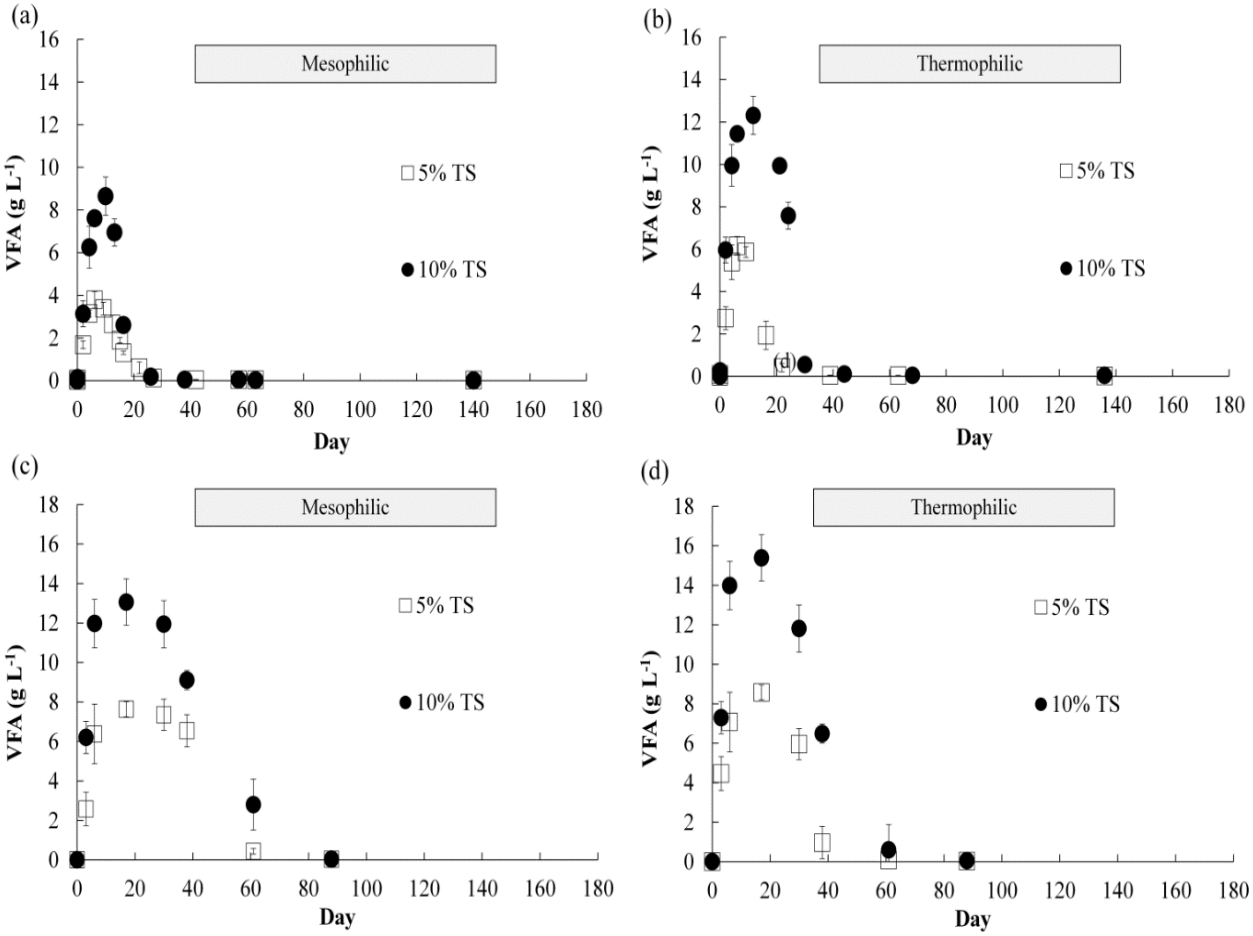

Figure 5.3. VFA profile during batch digestion of spent bedding from Site C ((a); (b)) and D ((c); (d)) without external inoculation.

Methane production showed a substantial time lag, and the time lag was more pronounced for spent bedding from Site D than for bedding from Site C (Figure 5.4). Increasing TS resulted in a longer time lag. However, the time lag was generally shorter at 55 ˚C than at 35 ˚C (Table 5.4), indicating higher methanogenic activity at the higher temperature. The rate of methane production decreased with increasing TS, as indicated by a lower value k_{meth} at higher TS (Table 5.4). Methane production was generally insensitive to temperature (Table 5.4). However, process start-up had a higher 40 – 50 % chance of failure at 55 ˚C and 20 % TS, with start-up failure noted by minimal or no methane production (Figure 5.4). Further analysis of tests that had low methane yield, revealed that pH was depressed (~ 6.0 ± 0.5) by substantial VFA accumulation (12 to 16 g VFA L^{-1}), both of which could have inhibited methanogenesis.

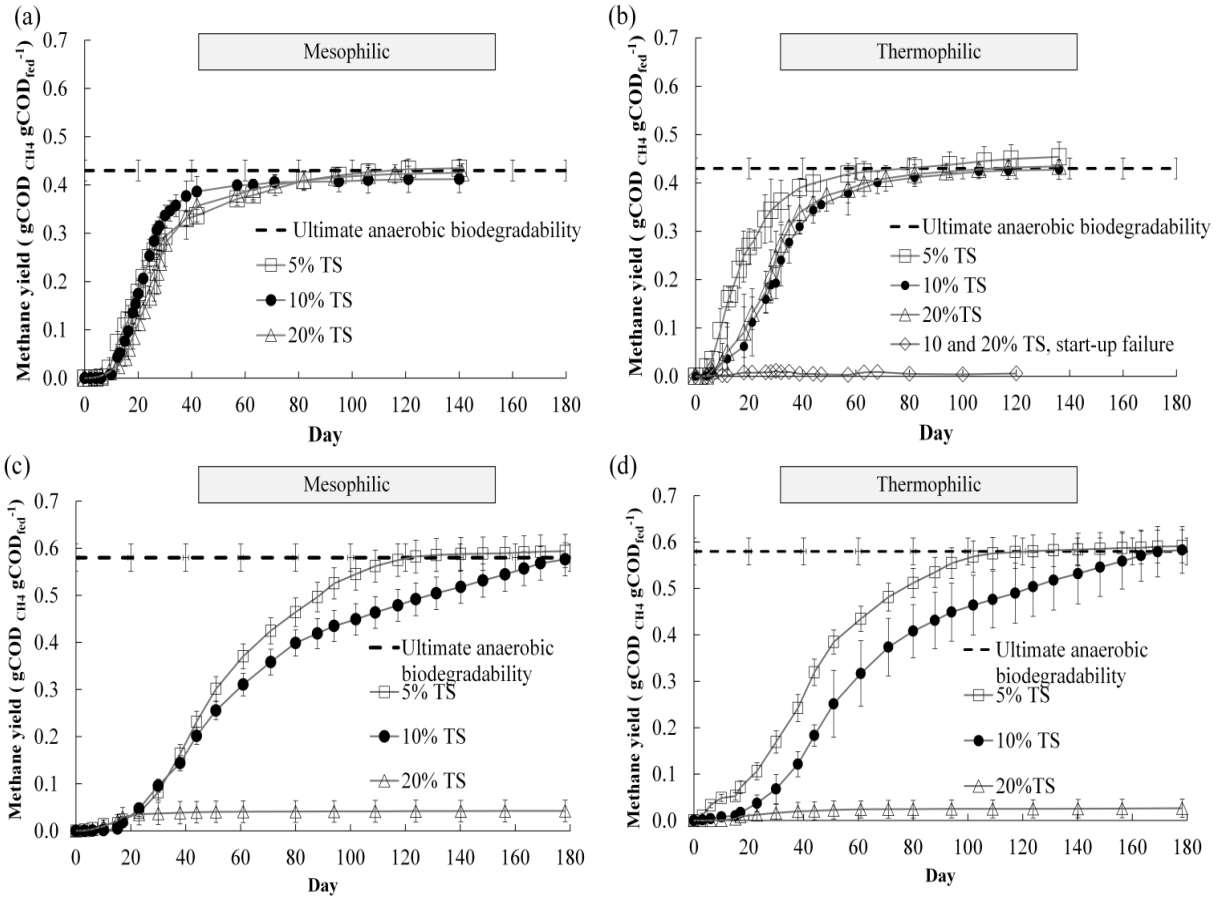

Figure 5.4. Cumulative methane production profile during batch digestion of spent bedding from Site C ((a); (b)) and D ((c); (d)) without external inoculation.

Overall, with 5 and 10 % TS at both mesophilic and thermophilic temperatures, it was possible to achieve the full biodegradability of each bedding substrate as measured by the BMP tests (Section 5.2.2). In all cases, the B_0 was recoverable for bedding from both Site C (140 ± 5 L CH_4 kg VS_{fed}^{-1}) and Site D (227 ± 6 L CH_4 kg VS_{fed}^{-1}) (Figure 5.4 and Table 5.4). The only exception was batch tests at 20 % TS for the Site D sample, which had a low methane production likely by inhibition and only 4-5 % of the B_0 value was recoverable after 180 days of digestion at these conditions.

Table 5.4. Model fit parameters for hydrolysis and methanogenesis for batch tests without inoculation (Section 5.2.2).

Spent bedding origin	T (˚C)	TS (%)	Hydrolysis		Methanogenesis		
			k_{hyd} (d^{-1})	f_d (%)	t_d (d)	k_{meth} (d^{-1})	Measured B_o (mL CH_4 gVS^{-1})
Site C	35	5	0.108 ± 0.004	39 ± 3	7.8 ± 0.5	0.075 ± 0.005	141 ± 5
		10	0.087 ± 0.004	42 ± 4	10.5 ± 0.2	0.068 ± 0.004	140 ± 4
		20	-	-	12.5 ± 0.4	0.053 ± 0.005	145 ± 8
	55	5	0.113 ± 0.004	42 ± 3	2.0 ± 0.4	0.078 ± 0.003	142 ± 4
		10	0.101 ± 0.004	41 ± 3	4.5 ± 0.3	0.071 ± 0.004	140 ± 5
		20	-	-	5.5 ± 0.5	0.056 ± 0.005	146 ± 12
Site D	35	5	0.055 ± 0.005	58 ± 3	12.0 ± 0.6	0.040 ± 0.002	231 ± 5
		10	0.042 ± 0.006	57 ± 2	16.1± 0.9	0.032 ± 0.005	225 ± 9
		20	-	-	20.0 ± 2.0	0.005 ± 0.001	12 ± 7
	55	5	0.057 ± 0.003	58 ± 2	4.8 ± 0.5	0.044 ± 0.003	230 ± 4
		10	0.035 ± 0.005	58 ± 3	13.2 ± 1.0	0.028 ± 0.002	223 ± 4
		20	-	-	23.0 ± 4.0	0.003 ± 0.001	9 ± 7

5.2.3 Reusing digestate and leachate as an inoculum

After the first set of batch tests were terminated (per section 5.1.2), leachate and solid residue were collected, analysed and reused as an inoculum for a subsequent batch test (Section 5.1.3). Table 5.5 summarises key characteristics of the collected leachate. Due to cost constraints, the concentration of humic substances could only be measured for leachate from the 5 % TS batch tests. The measured humic substance concentrations were 0.07 - 0.09 g L^{-1} in leachate from batch tests on Site C samples and 0.30 g L^{-1} in leachate from batch tests on Site D samples, respectively. DOM analysis showed that the leachate residue contained 10 - 20 %, 20 - 40 % and 50 - 60 % (of total dissolved carbon) of soluble microbial products, aromatic proteins and humic substance (including humic and fulvic-like acid), respectively. Total alkalinity for Site C and D leachates from the 5 % TS batch tests were 870 and 1100 mg $CaCO_3$ L^{-1}, respectively. With the microbial composition analysis on both the solid digestate and leachate residue, 30 to 40 % of the total DNA extracted was identified as archaea, dominated by *Methanosarcina*

Table 5.5. Characteristics of leachate residue from batch tests without inoculation.

Spent bedding origin	T (°C)	TS (%)	pH	TAN (gNH_4-N L^{-1})	Humic substance concentration (g L^{-1})
Site C	35	5	6.9 ± 0.2	0.3 ± 0.1	0.09 ± 0.01
		10	7.1 ± 0.3	0.6 ± 0.1	n/a
		20	7.3 ± 0.2	1.3 ± 0.1	
	55	5	7.0 ± 0.3	0.4 ± 0.1	0.07 ± 0.01
		10	7.1 ± 0.2 / 5.9 ± 0.1*	0.7 ± 0.1	n/a
		20	7.2 ± 0.2 / 5.8 ± 0.3*	1.5 ± 0.2	
Site D	35	5	7.7 ± 0.2	0.7 ± 0.1	0.31 ± 0.02
		10	7.9 ± 0.3	1.7 ± 0.2	n/a
		20	6.0 ± 0.1	3.3 ± 0.1	
	55	5	7.8 ± 0.2	0.6 ± 0.1	0.29 ± 0.01
		10	7.7 ± 0.2	1.5 ± 0.2	n/a
		20	5.9 ± 0.2	3.4 ± 0.2	

*batch test with start-up failure

Figure 5.5 presents the results of the batch tests with inoculation. In all these tests, methane production begun near immediately, indicating that both the leachate and solid digestate had higher methanogenic activity and/or more suitable pH buffering than the original spent bedding (Section 5.2.2) for start-up of digestion. The rate of methane production was statistically identical in all these inoculated batch tests, regardless of whether they were inoculated with solid digestate or leachate (k_{meth} = 0.072 ± 0.002 d^{-1}). Dilution of leachate by 50 % with MilliQ water also did not significantly influence methane production rate, indicating that leachate had excess activity/capacity to prevent/overcome inhibition. In all the inoculated batch tests with Site C bedding, the B_0 was fully recovered within a test time of about 50 days (Figure 5.5a). With Site D bedding, digestion was slower (Figure 5.5b).

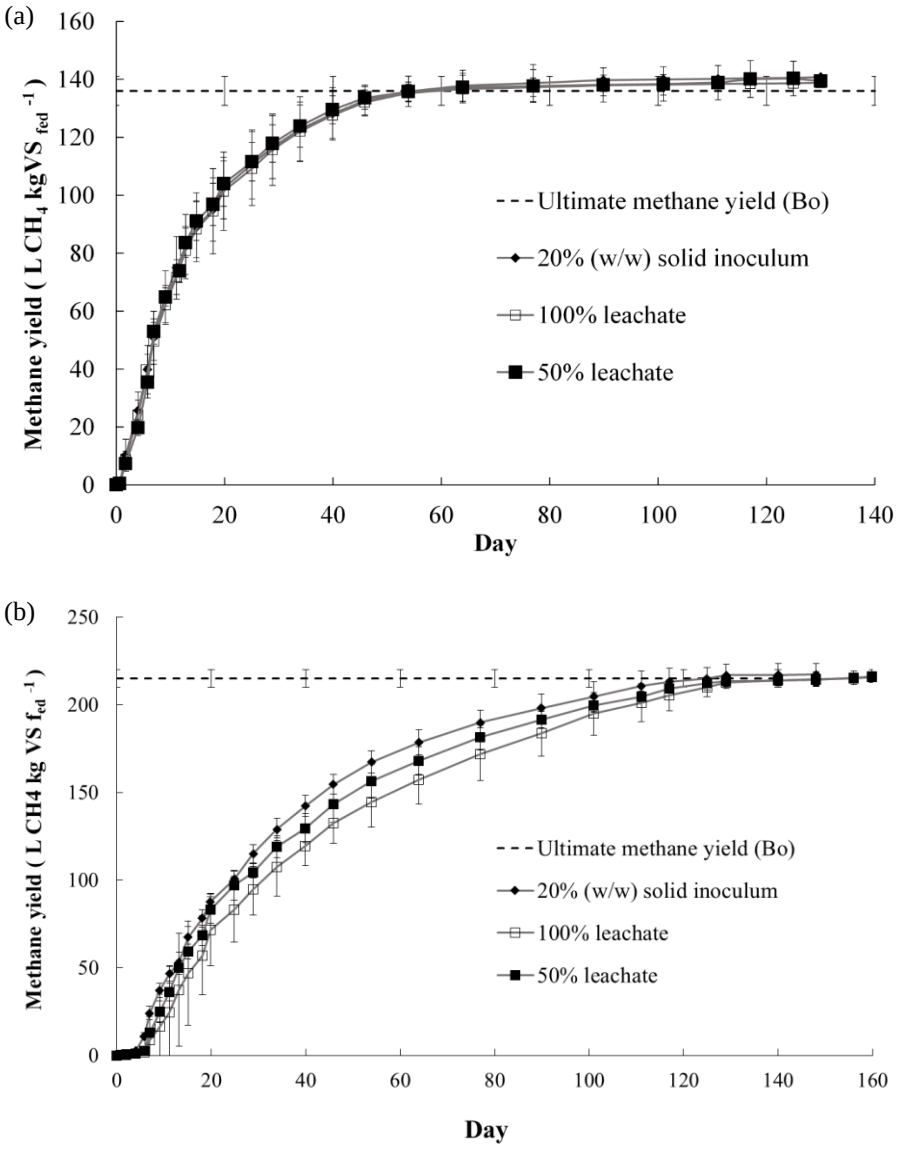

Figure 5.5. Methane production profile during batch digestion of spent bedding from (a) Site C and (b) Site D inoculated with solid digestate or leachate residue from previous at 10 TS% and mesophilic condition.

5.2.4 Implications

Inoculation strategies for AD start-up with solid manure residues

The test results (Section 5.3.1) showed that raw spent bedding contained adequate microbes for start-up of a solid-phase anaerobic digester. However, AD performance was different between the Site C sample and the Site D sample, with higher degradation rates for the site C sample. This could be due to in-shed exposure conditions at Site C, expected to favour growth of the indigenous microbial population. For example, extent of soilage, storage time and temperature were all lower at Site C. The methane yields were higher at Site D, possibly due to differences in the bedding material, or due to longer storage acting as pseudo pre-treatment.

The microbial analysis of fresh spent beddings (Figure 5.1) revealed the presence of hydrolytic bacterial groups that are known to degrade lignocellulosic material, and a low relative abundance of methanogenic archae in the spent bedding samples (Section 5.2.1). The relative abundance of methanogenic archaea, on the other hand, increased with digestion of the spent bedding (compare sections 5.2.1 and 5.2.3). It is possible that inadequate methanogenic archaea was initially present in the spent bedding to prevent the VFA accumulation observed at the start-up of the batch tests (Figure 5.4). An increased relative abundance of archaea in digestate as an inoculum appeared to expedite start-up of digestion, with a near-immediate onset of methane production (Figure 5.5). Both solid digestate and leachate recycled from a previous batch digestion were shown to be effective and consistent inoculums for start-up of subsequent batch digestion. However, leachate would likely be preferred over solid residue as an inoculum, to preserve treatment capacity for fresh waste (Section 2.4.2). Inhibitors could accumulate in leachate with multiple reuses and thus impact on digestion performance (Shahriari et al., 2012; Yap et al., 2016), and this is further addressed in Chapter 6.

Influence of temperature on microbial activity and digestion performance

Digestion performance was mostly comparable at mesophilic and thermophilic conditions (Section 5.2.2), with similar k_{hyd} and k_{meth} values (Tables 5.5). B_0 and f_d were also not significantly different at the two operating temperatures. However, the risk of start-up failure at 10 and 20% TS was higher at the thermophilic condition (55 ˚C) than at the mesophillic condition (35 ˚C). This could have been caused by (1) an inconsistent level of suitable

methanogenic archaea (Hori et al., 2006) and/or (2) faster rates of hydrolysis and fermentation (Figure 5.2) typically accompanied by a depressed pH (Li et al., 2010). In isolation, these results would suggest that mesophilic start-up is preferred over thermophilic start-up, given the higher failure risk at thermophilic conditions. The key issue is sourcing an acclimatised inoculum to ensure a quick and successful thermophilic start-up (De la Rubia et al., 2012). Such an inoculum may not be readily available. Future work can explore digestion performance under thermophilic conditions using inoculum pre-acclimated to thermophilic conditions.

Effect of solids loading on indigenous microbial performance

The test results suggested that dissolved organic compounds and intermediates partially inhibited the AD at higher TS. Specifically for ammonia as an inhibitor, TAN concentration in the tests at 10 and 20 % TS (Table 5.5) was at levels where both hydrolysis and methanogenesis could be significantly inhibited (Chen et al., 2008; Wilson et al., 2013). Albeit that at 10 % TS, no VFA accumulation was detected once methane production had commenced (Figure 5.3 c, d), indicating that ammonia was low enough to prevent substantial inhibition. With respect to recalcitrant organics, DOM analysis revealed that the aqueous phase contained a considerable amount of humic/fulvic acid compounds (Section 5.2.3). While for the batch tests at 5 % TS, the measured concentration of humic substances was at the lower end of reported inhibitory values (Brons et al., 1985; Fernandes et al., 2014; Ghasimi et al., 2016), their concentration would have been notably higher in batch tests at 10 and 20 % TS. Humic substances are said to influence hydrolysis but not methanogenesis (Fernandes et al., 2014; Ghasimi et al., 2016), and this is further explored in Chapter 6. Inhibition by HA and ammonia is particularly important when leachate is recycled as a secondary inoculum for subsequent digestion (Section 5.2.3), thereby progressively accumulating inhibitors. This aspect is also addressed in Chapter 6.

CHAPTER 6
Understanding inhibition by humic acid (HA)

Ammonia and humic acid (HA) are both potential inhibitors in solid-phase AD of manure residues (Chapter 5). However, while ammonia is well-documented as an inhibitor (Section 2.4.3), much less research attention has been given to HA. The mechanisms of HA inhibition are also a subject of on-going research (Section 2.4.3). The mitigation of HA inhibition is currently limited to physico-chemical means. However, there may be opportunity to overcome inhibition by using the inherent microbial resilience in an inoculum (Section 2.4.3) This chapter examined the impact of HA on hydrolytic and methanogenic activity between concentration ranges of 0 – 20 g L^{-1}. The impact of microbial community composition and microbial activity on HA inhibition was investigated by using inoculums. These inoculums were sourced from distinct anaerobic digesters with different feed types, as a selector for microbial community differences. The study aimed to better understand inhibition mechanisms for HA and to determine relationships with microbial activity and community composition.

6.1 Materials and Methods

6.1.1 Materials

All substrates were analytical reagent grade and purchased from Sigma-Aldrich. Sodium acetate anhydrous, gelatine and α-cellulose were added to represent acetate, protein and carbohydrate substrates, respectively. The inhibitor HA was added as a sodium salt (lot number 16308-048, CAS number 68131-04-4). Four inoculums were studied, namely:

- DSS: digestate from a 5500 m^3 mesophilic digester (35 ˚C) at a domestic wastewater treatment plant in South East Queensland, treating a mixture of primary and secondary sludge at a hydraulic retention time (HRT) of 23 - 24 days.

- THD: digestate from a mesophilic (37 ˚C) anaerobic digester at a centralised municipal biosolids processing facility in South East Queensland, fed with thermally hydrolysed sludge from a CAMBI® process (155 ˚C and 4.5 bar).

- PLS: sludge extracted from the base of a covered anaerobic lagoon treating coarse-screened flush manure from grower-finisher pig sheds in Victoria, Australia. The

sludge was extracted using a vacuum tanker connected to sludge extraction ports through the side banks of the lagoon near the inlet side of the lagoon.

- PPDS: digestate from a completely mixed tank digester located at a piggery in Queensland Australia, treating a mixture of macerated paunch from a nearby abattoir together with pig flush manure, at 25 ˚C and a HRT of about 15 days. The facility produced an about 130 kWe and 70 kWe from digestion of the manure fraction and paunch fraction, respectively.

The inocula were characterised for pH, color (mg PtCO L^{-1}), TS,VS, tCOD/sCOD, VFAs and TAN (Section 3.4). Chemical composition is summarised in Table 6.1. Microbial community composition of each inoculum was characterised as per Section 3.7.

Table 6.1. Physical characteristic of inoculums used in inhibition testing.

Parameter	DSS	THD	PPDS	PLS
pH	7.00 ± 0.03	6.98 ± 0.03	7.92 ± 0.01	6.99 ± 0.04
TS (g kg^{-1})	30 ± 2	49 ± 3	28 ± 2	52 ± 3
VS (g kg^{-1})	21 ± 2	31 ± 3	19 ± 2	40 ± 2
VS/ TS (%)	71	61	69	77
tCOD (g L^{-1})	33 ± 3	53 ± 4	41 ± 4	64 ± 4
sCOD (g L^{-1})	0.2 ± 0.1	5.2 ± 0.4	2.8 ± 0.1	0.6 ± 0.1
VFAs (mg L^{-1})	61 ± 10	97 ± 22	445 ± 111	87 ± 18
TAN (mg NH4-N L^{-1})	210 ± 12	2665 ± 70	1648 ± 12	612 ± 36
Color (mg PtCO L^{-1})	2667	13581	2558	10490

6.1.2 Inhibition tests

Two sets of inhibition testing were conducted, with each inhibition test set consisting of a hydrolytic activity test and a specific methanogenic activity (SMA) test as described below. In Set 1, all four inoculums were tested with HA at concentrations between 0 to 2 g L^{-1}. In Set 2, only DSS was tested for a broader range of HA concentrations of 0 to 20 g L^{-1}. The inhibition tests were conducted as short-term batch experiments, so that the microbial community in the test was predominantly represented by the microbial community of the added inoculum.

Hydrolytic activity test

Hydrolytic inhibition tests were performed in 160 mL glass serum bottles (working volume 100 mL) at 37 ± 1˚C, according to the method of Astals et al. (2015). This included pre-dilution of inoculums to 10 g VS L^{-1} using MilliQ water to minimize interference by background inhibitors. Cellulose or gelatine were added in all cases at an ISR of 5 on a VS basis. Prior to the test, the inoculums were stored at 37 ± 1 ˚C for 5 days to de-gas.

Six replicate bottles were run. During the course of the test, 1 mL of liquid samples were withdrawn intermittently from three of the six test bottles, while biogas samples were collected for analysis from the remaining three bottles. Soluble fractions were analysed for VFAs and sCOD as per Section 3.4. Background methane production from substrate-free blanks were subtracted from the cumulative methane produced by the test batches. Tests were mixed by inverting once before every sampling event, but not between sampling events. Biogas volume was measured using a displacement manometer as described by Jensen et al. (2011) and biogas composition determined as per Section 3.6. The batch experiments were terminated when the net methane produced was insignificant (<1% of the cumulative methane produced up to the last of those three days) (Holliger et al., 2016).

SMA

SMA was determined according to the method of Astals et al. (2015). The inoculum to acetate ratio was set at 5.0 g VS g $acetate^{-1}$, with each inoculum being pre-diluted to 10 g VS L^{-1}. Cumulative methane production was determined using a displacement manometer and GC as described above.

6.1.3 Data analysis

The package Aquasim 2.1d (Jensen et al, 2011) was used to analyse data for net cumulative methane produced (B_t) over time (t) for cellulose and gelatine as substrates and for each inoculum. The analysis performed a non-linear least-squares fit with a simple first-order kinetic model with a lag phase (t_d, units of d, a fitted parameter) (Equation 6.1):

$$B_{t,i} = \begin{cases} 0\,, & and \quad t < t_d \\ B_{o,i}\left(1 - e^{-k_{hyd} \cdot t}\right), & t \geq t_d \end{cases} \qquad (6.1)$$

where $B_{0,i}$ is the measured maximum methane yield (unit of g COD g VS_{fed}^{-1}). Subscript i is either ch or pr representing cellulose and gelatine, respectively.

SMA was determined as the slope of a linear regression fit (analysis Toolpak in Microsoft Excel 2010) to the cumulative methane produced over time on acetate as substrate, and as described by Astals et al. (2015).

For microbial community analysis, OTU tables were normalised and a square root transformation was applied to emphasise comparison of niche populations over dominants. A principal component analysis (PCA) was performed according to the method described by Batstone et al. (2015).

6.2 Results and discussion

6.2.1 Methanogenesis

The results showed that HA concentrations of between 0 - 2 g L^{-1} did not have a significant effect on methanogenic activity, with measured SMAs of the four inoculums (DSS, THD, PLS and PDDS) being comparable at 0.109, 0.92, 0.95 and 0.75 g COD g $VS_{inoculum}^{-1}$ d^{-1}, respectively. These findings were in agreement with a previous study (Ghasimi et al. (2016)) with HA concentrations up to 2.0 g L^{-1}. However, when HA concentration was further increased to above 5 g L^{-1}, there was significant inhibition of methanogenic activity (Figure 6.1). Methanogenesis was completely inhibited at a HA concentration of 20 g L^{-1} (Figure 6.1). Whilst the addition of the HA salt at 20 gL^{-1} HA would have corresponded to the simultaneous addition of 2 gL^{-1} sodium, inhibition of acetoclastic methanogenesis by sodium more commonly occurs at much higher concentrations > 3 gL^{-1} (Astals et al., 2015; Chen et al., 2008; Feijoo et al., 1995). The observed inhibition was therefore more likely caused by HA. HA inhibition of acetoclastic methanogenesis has not been previously reported, but HA concentrations in other studies have been much lower (Brons et al., 1985; Fernandes et al., 2014; Ghasimi et al., 2016). All the inoculums were dominated by the acetoclastic methanogen *Methanosaeta* (relative abundance between 57 to 66%). Similar to the present study, previous works by Khadem et al. (2017) and Ghasimi et al. (2016) have observed that *Methanosaeta* can withstand HA concentrations up to 3 g L^{-1}. The study by Khadem et al. (2017) have suggested that *Methanosaeta* has better resistance to HA inhibition when compared to other methanogens, because of the cell wall structure of *Methanosaeta*.

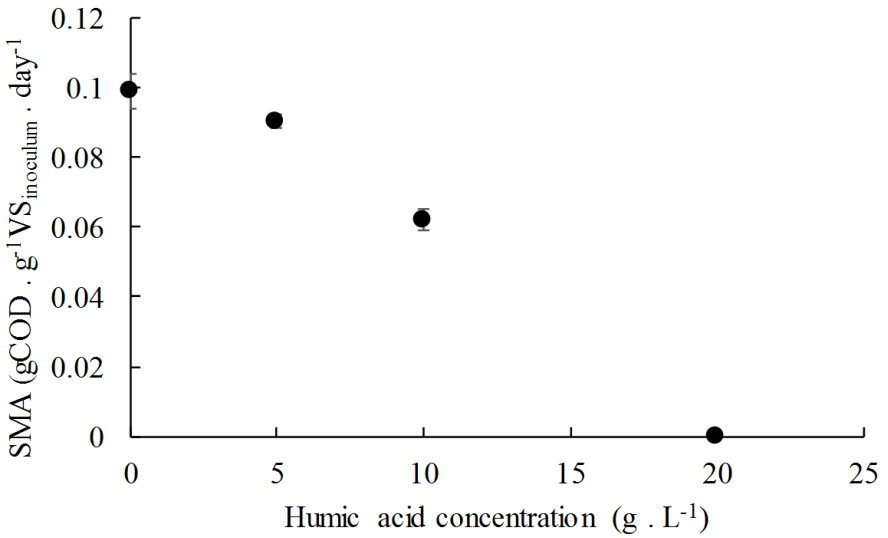

Figure 6.1. Methanogenic activity (SMA) of DSS at humic acid concentration range between 0 - 20 g L^{-1}.

6.2.2 Hydrolysis

Low HA concentration (0 – 2 gL^{-1})

For THD and PDDS as inoculums, cellulose degradation was affected by HA over the range 0 – 2 g L^{-1} (Table 6.2). For these inoculums, the presence of HA induced a greater lag phase t_d than in the control tests and the lag phase was longer at higher HA concentrations. Interestingly, t_d increased linearly with increasing HA concentration for these inoculums (THD and PDDS) and cellulose (Figure 6.2). For DSS and PLS, t_d was significant, but was not significantly different from that of the control tests at HA concentrations up to 2 g L^{-1} (Table 6.2). After the t_d, the kinetics k_{ch} and B_0 (~ 0.79 g COD g VS_{fed}^{-1}) of cellulose digestion was not significantly influenced by increasing HA concentration up to 2 g L^{-1}. These results suggested that bacterial activity was not affected by HA up to 2 g L^{-1}, because influence on bacterial activity would have caused a decrease in k_{ch} values. This contrasts with mechanisms suggested by Smith et al. (2005), i.e. humic substances binding to microbial cell walls. Analysis of soluble fractions revealed no accumulation of VFA or sCOD for HA up to 2 g L^{-1}, indicating that methanogenesis was not rate-limiting.

Unlike cellulose, protein degradation was not significantly influenced by the presence or concentration of HA up to 2 g L^{-1}, with no significant lag phase (i.e. not significantly different from zero) and with no significant impact on k_{pr} or B_0 (~0.85 g COD g VS_{fed}^{-1}) (Table 6.3). In contrast, Brons et al. (1985) found that HA inhibited protein hydrolysis at concentrations as

low as 0.25 g L^{-1}, but humate compositions and microbial community composition (see below) could have been different than in the present study. Future testing should consider different humate compositions (e.g. humic vs. fulvic acid) for particular microbial communities to elucidate the relative impact of humate composition

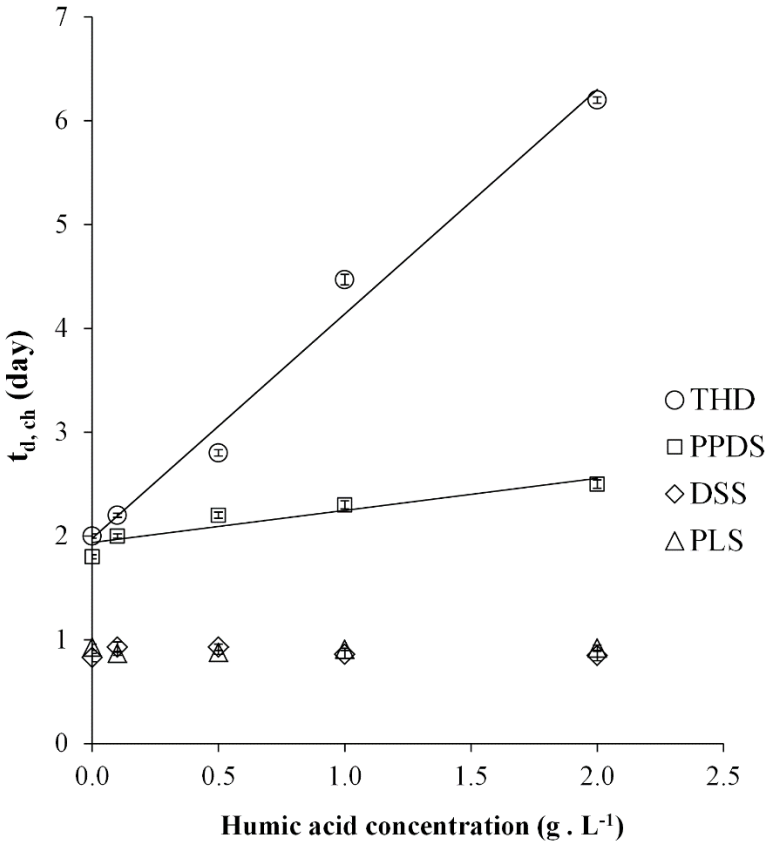

Figure 6.2. Cellulose degradation start-up ($t_{d,ch}$) over humic acid concentration range of 0 - 2 g L^{-1}

High HA concentration (0 – 20 gL^{-1})

For DSS as inoculum and at higher HA concentrations of 5 g L^{-1} and above, hydrolytic activity appeared to be affected for both cellulose and gelatine. As seen in Figure 6.3, t_d became significantly longer whilst degradation rates (k_{ch} and k_{pr}) also decreased significantly with increasing HA concentrations from 5 to 20 g L^{-1}. At HA concentration between 5 and 10 g L^{-1}, methanogenesis was not expected to be rate limiting, because no sCOD or VFA accumulation was detected. However, at a HA concentration of 20 g L^{-1}, VFA did accumulate and the measured methanogenic activity on acetate was essentially nil (Figure 6.1), so at this condition methanogenesis could have been rate limiting.

Table 6.2. Model outputs for cellulose and gelatine hydrolysis using different inoculums at HA concentration range between 0 – 2 g L^{-1}.

HA level	DSS				PLS				THD				PDDS			
	Cellulose degradation		Gelatine degradation		Cellulose degradation		Gelatine degradation		Cellulose degradation		Gelatine degradation		Cellulose degradation		Gelatine degradation	
	t_d	k_{ch}	t_d	k_{pr}	t_d	k_{ch}	t_d	k_{pr}	t_d	k_{ch}	t_d	k_{pr}	t_d	k_{ch}	t_d	k_{pr}
(g L^{-1})	(d)	(d^{-1})	(d)	(d^{-1})	(d)	(d^{-1})	(d)	(d^{-1})	(d)	(d^{-1})	(d)	(d^{-1})	(d)	(d^{-1})	(d)	(d^{-1})
0.0	0.83 ± 0.04	0.67 ± 0.02	0.0	0.62 ± 0.02	0.93 ± 0.03	0.64 ± 0.03	0.0	0.61 ± 0.03	2.01 ± 0.02	0.49 ± 0.03	0.40 ± 0.02	0.53 ± 0.03	1.80 ± 0.02	0.53 ± 0.03	0.0	0.62 ± 0.03
0.1	0.93 ± 0.05	0.68 ± 0.03	0.0	0.62 ± 0.02	0.87 ± 0.02	0.66 ± 0.02	0.0	0.60 ± 0.04	2.20 ± 0.02	0.47 ± 0.02	0.38 ± 0.03	0.55 ± 0.02	2.00 ± 0.02	0.53 ± 0.03	0.0	0.61± 0.02
0.5	0.93 ± 0.03	0.68 ± 0.03	0.0	0.64 ± 0.02	0.88 ± 0.02	0.64 ± 0.03	0.0	0.59 ± 0.02	2.80 ± 0.03	0.51 ± 0.04	0.37 ± 0.04	0.55 ± 0.03	2.20 ± 0.03	0.54 ± 0.02	0.0	0.61 ± 0.02
1.0	0.86 ± 0.04	0.68 ± 0.02	0.0	0.61 ± 0.02	0.91 ± 0.01	0.64 ± 0.03	0.0	0.59 ± 0.02	4.70 ± 0.05	0.48 ± 0.03	0.38 ± 0.02	0.55 ± 0.04	2.30 ± 0.04	0.52 ± 0.03	0.0	0.61 ± 0.02
2.0	0.95 ± 0.05	0.68 ± 0.02	0.0	0.62 ± 0.02	0.92 ± 0.03	0.66 ± 0.03	0.0	0.58 ± 0.03	6.20 ± 0.03	0.48 ± 0.03	0.34 ± 0.03	0.56 ± 0.02	2.50 ± 0.04	0.52 ± 0.03	0.0	0.61 ± 0.03

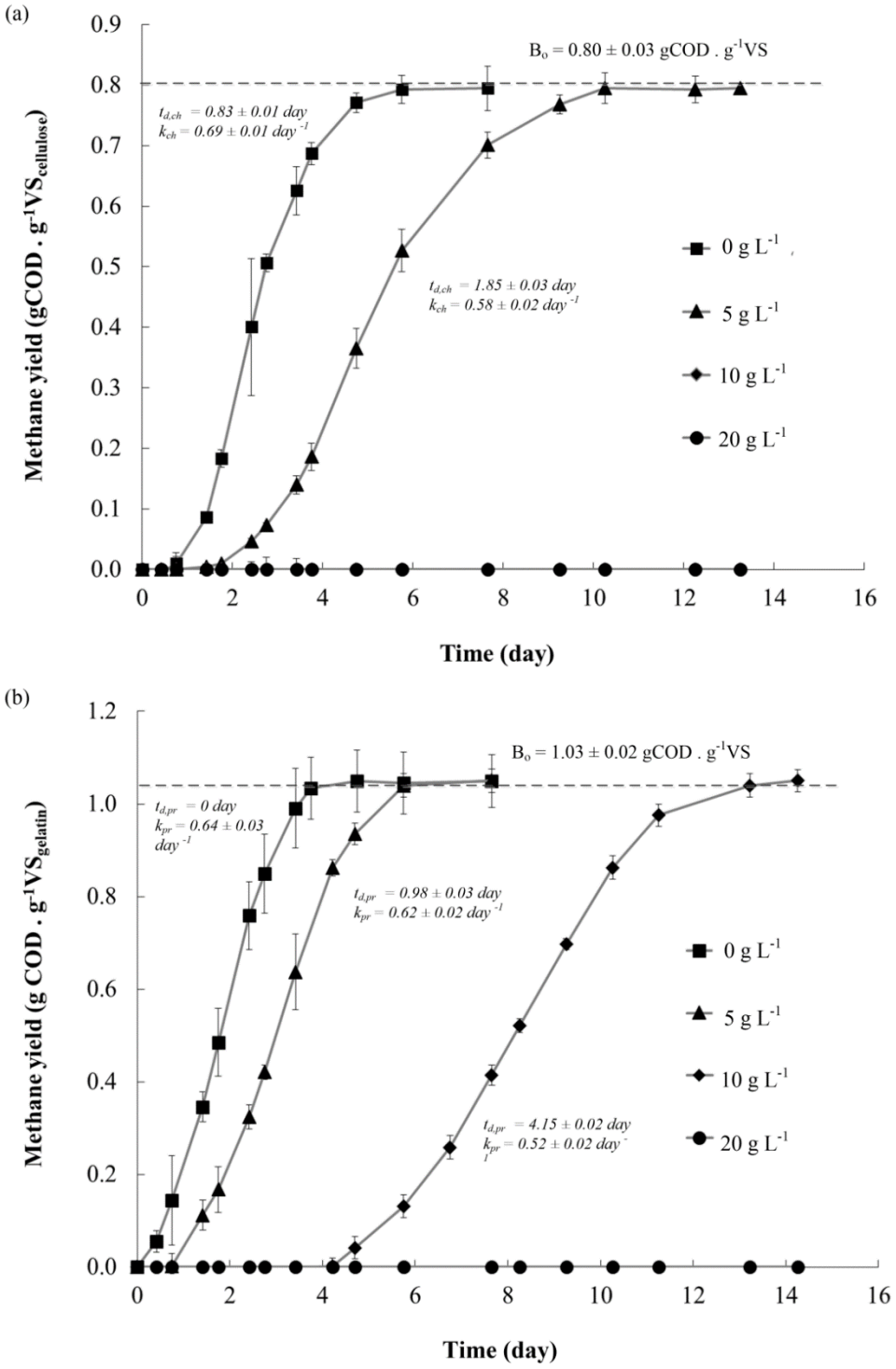

Figure 6.3. Hydrolytic activity plot and model outputs for (a) cellulose and (b) gelatine hydrolysis using DSS at humic acid concentration range between 0 - 20 g L^{-1}.

Inhibition mechanisms

A mechanistic hypothesis is stated here to interpret the observations for cellulose digestion at HA up to 2 gL^{-1}:

- Bacteria excrete enzymes at a specific rate to hydrolyse cellulose, however HA complexes with the enzymes, preventing them from binding with cellulose.

- Bacteria continue to excrete enzymes at a specific rate (based on microbial density and excretion capacity). At higher HA concentrations a longer lag-phase (t_d) results, because it takes longer for all HA to complex with excreted enzymes.

- Subsequent to the lag-phase (t_d), enzymes continue to be excreted and are then available to bind with cellulose. Hydrolysis then proceeds unaltered by complexed HA, and the rate of hydrolysis is dictated by enzyme excretion and enzyme reaction.

Further testing is required, but the mechanism proposed above appears to be in good agreement with observations of the present study, and with results from previous studies where enzymatic activities during substrate degradation were reduced with increasing HA concentrations (Fernandes et al., 2014; Li et al., 2013). Previous studies have also demonstrated that bacterial activity was positively correlated with enzyme production (Nybroe et al., 1992). It is possible that DSS and PLS in the present work had more abundant free enzymes or higher enzyme excretion rates than THD and PDDS, and therefore was able to more rapidly overcome inhibition by HA.

The mechanism of HA inhibition at > 5 g L^{-1} appears to be different than for HA up to 2 g L^{-1}. HA inhibition at the higher concentrations was perhaps a combination of higher order complexing of substrates, enzymes and HA, as proposed by Tomaszewski et al. (2011) and Li et al. (2013), together with inhibition of methanogenic activity (Section 6.2.1). In these regards, cellulose degradation could be more susceptible than protein degradation, because of selective inhibition for specific substrates (Tan et al., 2008) and/or specific enzymes or targeted impacts on microbial communities (see directly below).

PCA of the bacterial community indicated occupancy of one of three major spaces (Figure 6.4). The more resilient DSS and PLS inoculums (no increase in t_d with increasing HA up to 2 gL^{-1}) occupied the upper left quadrant, associated with increased relative abundance in

the phyla *Bacteriodetes*, *Chloroflexi*, *Planctomycetes* and *Proteobacteria*. The less resilient THD and PPDS inoculums also exhibited lower microbial diversity than DSS and PLS. THD occupied the right hand region with a dominance of phylum *Firmicutes.* PPDS occupied the lower left region and was dominated by phylum *Spirochaetes* with a lesser contribution by *Tenericutes*. A closer examination of bacterial community composition (Figure 6.5) revealed the presence of bacterial strains of order *Plactomycetales; Sphingobacteriales* and *Syntrophobacterale* in the more resilient inoculums DSS and PLS, and these strains have been previously reported to be humic-resistant (Azman et al., 2016). However, while these differences in microbial community composition aligned with differences in HA resilience, *Plactomycetales; Sphingobacteriales* and *Syntrophobacterale* are not known to participate in hydrolysis (Muyzer and Stams, 2008; Scheurwater et al., 2008; van Teeseling et al., 2015) and hydrolytic bacteria with clear humic resistant properties could not be identified in the present study. Increased HA inhibition resilience may also result from higher microbial activity or microbial concentration, independent of differences in microbial community composition.

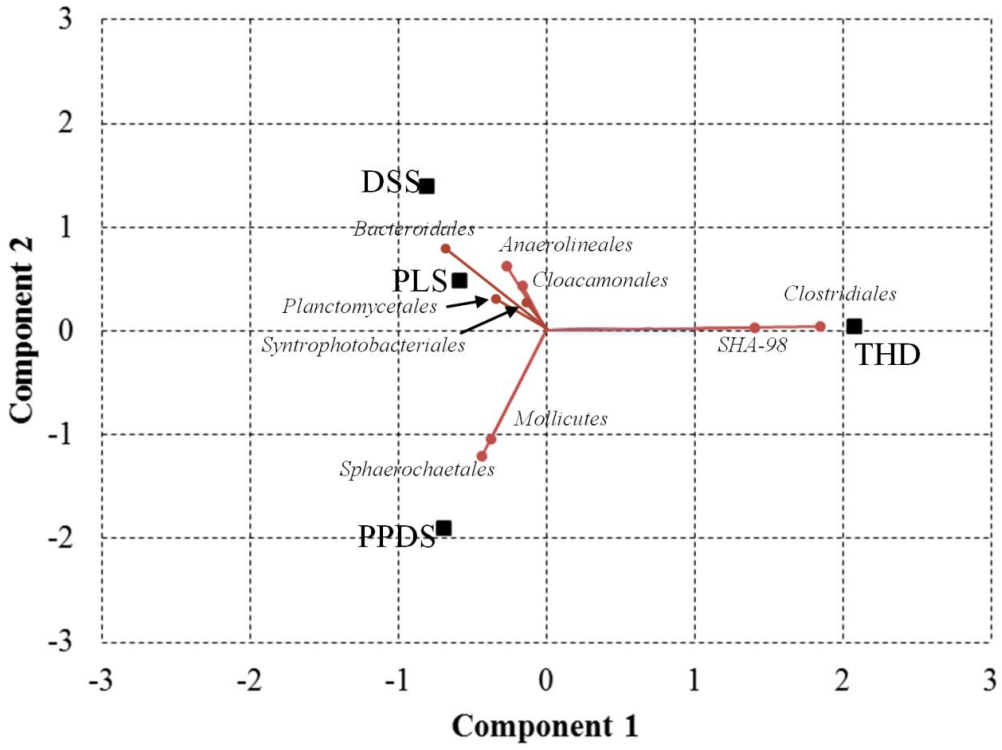

Figure 6.4: Principal Components analysis (PCA) of the bacterial population, indicating major vectors (red) and samples (black) in the PCA space.

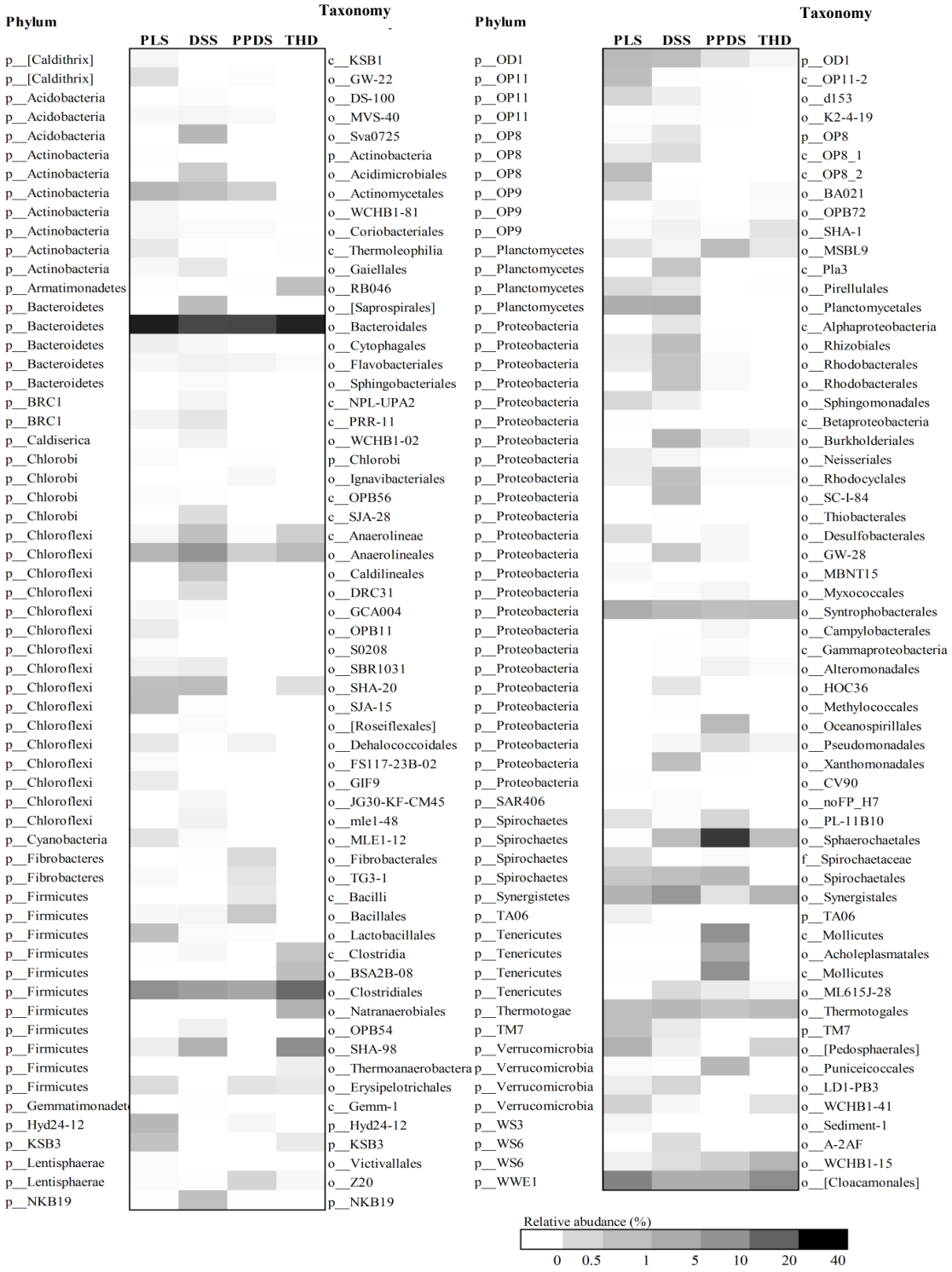

Figure 6.5. Bacterial composition of inoculums (>1% relative abundance). The heat map shows the relative abundance of microbial groups present in the inoculum. The taxonomic classification is shown at the phylum level (left-hand side) and phylum, class and order level of taxonomic assignment for bacteria (right-hand side), respectively, at 97% similarity.

6.2.3 Implications

The present study results demonstrated that different inoculums exhibit different HA inhibition susceptibility, possibly due to differences in bacterial activity (section 6.2.2). As the extent of recovery from HA inhibition was distinct for the different inoculums, these findings point towards the possibility of using inoculation as a source of inhibition resilience. However, no definitive link could be found between microbial community composition and resilience to HA inhibition. Therefore, it remains unknown whether the inhibition resilience is a property of specific hydrolytic bacterial strains, or rather due to microbial activity or microbial concentration differences. To address these unknowns, relevant recommendations for further work are stated in Chapter 7.

CHAPTER 7
Conclusions and recommendations

This chapter summarises overall implications and significance of the research findings relative to the stated thesis objectives (Section 1.2). It concludes with perspectives for future work and general remarks on applications of the research within an agricultural context.

7.1 Strategies to enhance leachbed performance

Leachbeds appear to be a highly attractive AD technology option for solid manure residues. However, to date leachbeds have mostly been used for the organic fraction of municipal solid waste and energy crops, with minimal studies with solid manure residues (Section 1.1). In general, leachbeds have been prone to poor performance with low methane yield. There appeared to be considerable opportunity to improve the performance of leachbeds via systematic research and development, targeting key strategies to promote AD in a leachbed.

7.1.1 Leachate contact to promote digestion in a leachbed

Process performance of leachbeds is heavily influenced by leachate contact and mass transfer, with leachate movement, hold-up and/or channelling in the solid bed being key considerations. From an applications perspective, short-circuiting or pooling/trapping of leachate are key concerns (Mussoline, 2013). Two approaches have been previously investigated to facilitate mass transfer and contact with leachate, namely trickling leachate flow and flood-and-drain operation. When leachate is trickled, an increase in leachate recirculation rate could increase mass transfer (Vavilin et al.,2002) and improve AD performance (Chugh et al., 1998; Veeken and Hamelers, 2000; Vavilin et al., 2002), but may not resolve leachate channelling (Morris et al., 2003). A flooded leachbed would enable the solid bed to be fully submersed with more intimate contact with leachate (Nizami et al., 2010) and leachate flow direction can be reversed to improve the porosity of the solid bed, thus minimizing clogging and leachate chanelling (Uke and Stentiford, 2013).

The suitability of flood-and-drain vs. trickling operation has not been previously studied in parallel, nor for solid manure residues. This was done by the pilot work in Chapter 4, which examined these two different leachate flow configurations with solid manure residues (B_0 = 195 – 218 L CH_4 kg^{-1} VS_{fed}). Both trickling and flood-and-drain type leachbed had achieved

comparable methane conversion (50 % of the B_0), despite their markedly different leachate flow configurations (trickling versus flood-and-drain) (Section 4.2.2). However, the flood-and-drain leachbed mobilized about 30 % (g COD g COD_{fed}) more unwanted non-degradable particulates into leachate than the trickling leachbed (Section 4.2.4). This higher mobilization of particulates adds little methane (Section 4.2.5) and complicates leachate handling. As a result flood-and-drain leachbeds would likely require more maintenance than trickling leachbeds at full-scale. Also, at full-scale more water will likely be required for a flood-and-drain system than with a trickling system, due to the need for substrate to be fully submersed. Overall, the results in Chapter 4 indicated that a trickling arrangement would be preferred over a flood-and-drain arrangement.

Future work should explore other approaches or techniques to optimise digestion rate of solid manure residues in a leachbed system. For example, improving mass transfer within leachbeds via solid bed agitation (Vandevivere et al., 2003) or the addition of secondary bulking agents together with reduction of particle size to improve leachate access to the substrate (Mata-Alvarez et al., 2000; Yang et al., 2015).

7.1.2 Understanding microbial capability and determining possible inoculation strategies

Typically, external inoculum would be required for a balanced microbial population during leachbed start-up. However, external inocula may not be available for decentralised on-farm digestion in Australia, because many farms are very remote and have biosecurity restrictions limiting the flow of materials between farms. The manure component in spent bedding contains indigenous microbial community, which could self-inoculate the start up of a leachbed.

A limited knowledge of the indigenous microbial response to operating conditions of a leachbed, has impeded the development of effective inoculation strategies to improve leachbed performance. This is addressed in Chapter 5 through batch AD experiments at smaller laboratory scale, evaluating the effect of initial solids concentration, temperature and inoculation techniques on start-up and AD performance with solid manure residues. The results showed that the indigenous microbial activity in solid manure residues can recover the full methane yield of solid manure residues (140 – 227 L CH_4 kg^{-1} VS_{fed}) (Section 5.3.2). This indicated that the solid manure residues itself could be a suitable inoculum source for

initiating AD in a leachbed, because of the indigenous microbial activity. To operate at higher solids loading (≥ 10 % TS) and higher temperatures, methanogenic activity could be boosted by inoculation with residue from a previous batch. The test results in Section 4.3.2 and 5.3.3 showed that recycling of solid digestate as an inoculant of a subsequent batch digestion accelerates leachbed process, with 50% shorter lag time during start-up. In Section 4.3.2, maximum VFA accumulation during leachbed start-up in a leachbed without inoculation was at least double that with inoculation, and this indicates that inoculation provides rapid on-set of a balanced microbial population. However, leachate residue would probably be preferred over solid digestate as an inoculum source, given the comparable process performance in tests using leachate or solid digestate as an inoculum, resulting in no delay during start-up and comparable AD rates (k_{meth} = 0.072 ± 0.002 d^{-1}) and methane yield (B_0 = 140 ± 6 L CH_4 kg^{-1} VS_{fed}) (Section 5.3.3). Also leachate inoculation would be less complex from an operations perspective and preserves the treatment capacity for fresh spent bedding (Cui et al., 2011; Zhu et al., 2015). Of key concern with multiple reuses of leachate is the potential for inhibitors to accumulate in leachate and thus impact on digestion performance (Section 5.4). In-line treatment or dilution of leachate with process water would likely be required to limit the accumulation of inhibitors and thereby maintain digestion performance.

Future work should explore alternate liquid or effluent source streams on-farm as inoculant to start-up leachbeds (Section 4.3.5). This could be of interest to help manage the accumulation of inhibitors from recycling leachbed residues for inoculation. This is also to clarify relevant leachate properties, such as for example, microbial presence vs. alkalinity.

7.1.3 Strategies to limit or overcome chemical inhibition

Leachbed performance would be susceptible to chemical inhibition. This is due to elevated inhibitor concentrations at high solids loading and with the reuse of leachate to conserve water or inoculate. In Chapter 4, the leachbed trials were only capable of recovering 50 % of the B_0 and the ensuing post-digestion tests suggested that the trial performance could be hindered by either lack of suitable microbial community to fully digest the substrate or by chemical inhibition. Batch experiments in Chapter 5, which tested indigenous microbial capability for self–inoculation, showed that the microbial community was able to recover full methane potential of substrate; thus indicating that the performance of pilot-scale leachbeds was rather limited by chemical inhibition. Post-digestion analysis in Chapter 5 suggested ammonia and HA as potential inhibitors in leachbeds treating solid manure residues. However, because ammonia inhibition in AD was well-documented as opposed to HA

inhibition (Section 2.5), Chapter 6 further examined the inhibition characteristics of HA at relevant concentrations and specifically looking at the impact of different inoculums.

In agricultural applications, HA inhibition of AD could be exacerbated by HA accumulation up to 5 gL^{-1} due to high proportions of organic matter (Fernandes et al., 2014). The results suggested that HA below 5 g L^{-1} (as in a young leachbed with fresh leachate) inhibits hydrolysis by complexing with hydrolytic enzymes, leading to a delay during process start-up between 1 to 4 days. Between HA concentration of 5 and 20 g L^{-1}, both hydrolytic and methanogenic activity significantly decreased with increasing HA concentration, but hydrolysis appeared to be the rate limiting step for up to 20 g L^{-1} HA (Chapter 6). At HA of 20 g L^{-1}, AD activity was completely inhibited. Unfortunately, this high HA concentration could be relevant with multiple uses of leachate to start-up leachbed reactors. Interestingly, selective inhibition by HA was observed, with AD of a carbohydrate particulate (cellulose) being more susceptible to inhibition than AD of a protein (gelatine). This is relevant for co-digestion mixtures.

The results in Chapter 6 also demonstrated that different inoculums exhibit different HA inhibition susceptibility, possibly due to differences in bacterial activity. As the extent of recovery from HA inhibition was distinct for the different inoculums, these findings pointed to the possibility of using inoculation as a source of inhibition resilience. However, no definitive link could be found between microbial community composition and resilience to HA inhibition. Therefore, it remains unknown if the inhibition resilience is a property of specific hydrolytic bacterial strains, or rather due to microbial activity or microbial concentration differences.

Future studies should examine whether microbial communities could acclimatise to humic substances, without comprising AD activity. Such studies could consider continuous digestion in the presence of constant HA levels, to monitor microbial population dynamics and assess impacts on AD performance (i.e. hydrolytic and methanogenic activity). This could provide useful information to inform mitigation strategies with AD of solid manure residues.

7.2 Concluding remarks

The work of this thesis highlighted the potential for leachbed digestion of solid manure residues to recover energy (~ 50 % of B_0) on farm. The research clarified operational factors as a means to improve and ultimately optimize leachbed digestion performance, including modes of leachate recirculation (Chapter 4), inoculation techniques (Chapter 5) and microbial resilience to HA inhibition (Chapter 6). The study also future-proofed the thesis findings by investigating the impacts of relevant key operating conditions for leachbeds, such as solids loading and temperature (Chapter 5). Integration of leachbed technology into current waste management practice on-farm was proposed at the end of Chapter 4 based on the pilot leachbed studies. It is anticipated that leachbed could readily form part of the future AD technology for solid manure residues, especially if chemical inhibition can be effectively managed through microbial acclimatization to induce inhibition resilience (Chapter 6).

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APPENDIX A: Preliminary trial of batch system.

A test trial was conducted at pilot scale with a preliminary single stage setup, as illustrated in Figure A.1:

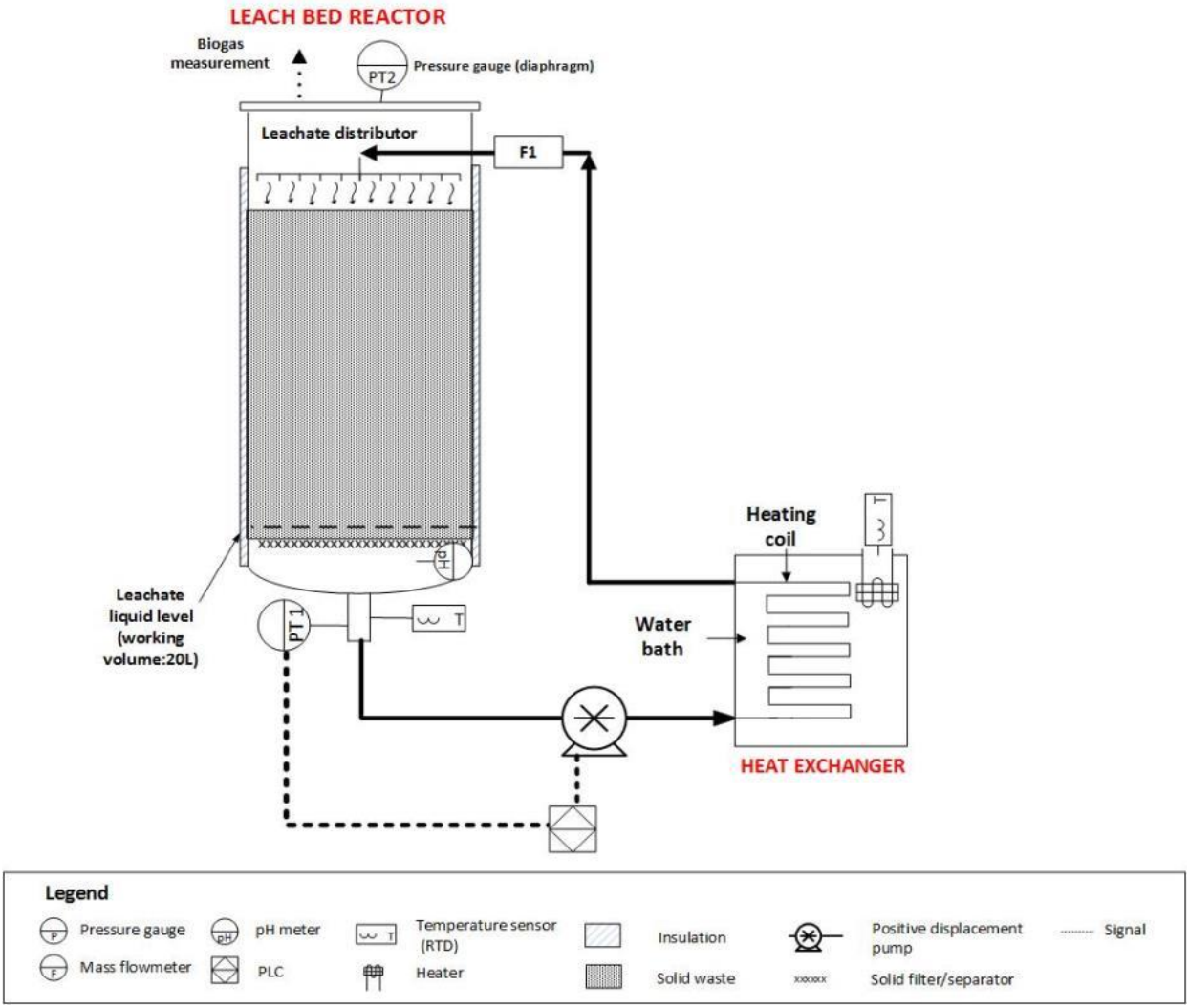

Figure A.1. Preliminary design of single stage batch system.

The aim of the experiment is to study the effect of inoculation on the process start-up performance of single stage batch trickling system at mesophilic condition (35 ˚C). The experimental condition was as Table A.1.

Table A.1. Experimental condition of single stage batch system

Parameter	LBR1	LBR2
Substrate – spent litter* (kg)	30	30
Inoculum – pig slurry ** (kg)	9	0
Inoculum to substrate ratio (VS)	0.11	0
TS (%)	10.71	9.28
Leachate recirculation strategy	Leachate is recirculated and trickled onto the solid residue bed for 5 minutes every 20 minutes	

*26 %TS, 21 % VS; ** 15 % TS, 7.5 % VS

The experiment was discontinued due continual clogging of spiral cone sprinkler from straw (as indicated by pressure build-up in the process line) as well as flooding and liquid hold-up in solid bed (See figure A.2 and 3), which led to shortage of available leachate for recirculation. These problems had also led to ineffective control of temperature which is a key parameter to ensure efficient anaerobic digestion. The data that were collected from this trial were not sufficient and consistent enough for any conclusion to be made. Nevertheless, the trial highlighted two key issues:

the sprinkling of liquid over the bed of litter and decay of the bed of litter over time was rapidly causing compaction of the bed, and eventually led to pooling of liquid on the bed suggesting poor hydraulics. The implications for an on-farm system would be that a large quantity of liquid will collect on the leachbed to ultimately force liquid through the bed by the static head pressure of the liquid pooled on the bed; and

two different sprinkler arrangements (a four rod-cross sprinkler, and a spiral pig-tail sprinkler) were trialed, both of which became blocked with fibrous spent bedding material that carried over from the decaying solid bed (Figure A.4).

These observations highlighted practical issues that a farmer may experience when the technology is applied on-farm, and thus required a rethink of the leachbed design. These problems have been taken into consideration during the refurbishment of the batch system for the subsequent trials (see section 2.6).

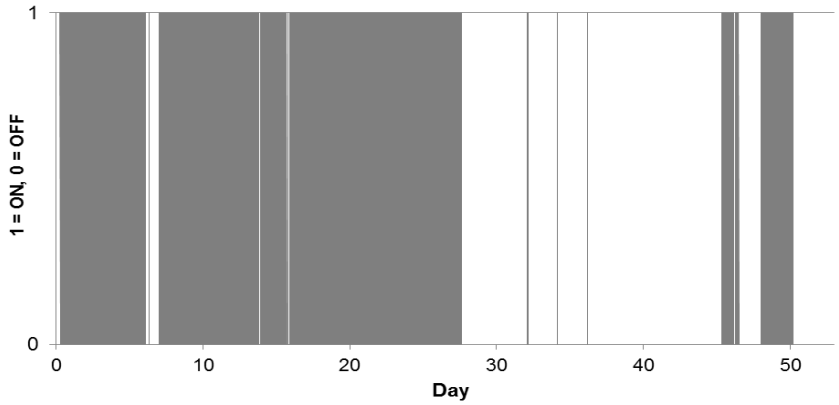

Figure A.2. Assessment of clogging in leachbed reactors via the leachate recirculation frequency (1= pump on, 0= pump off). Extended delay between leachate recirculation events indicated that leachate was retained in leachbeds, thus suggested possible clogging in the system.

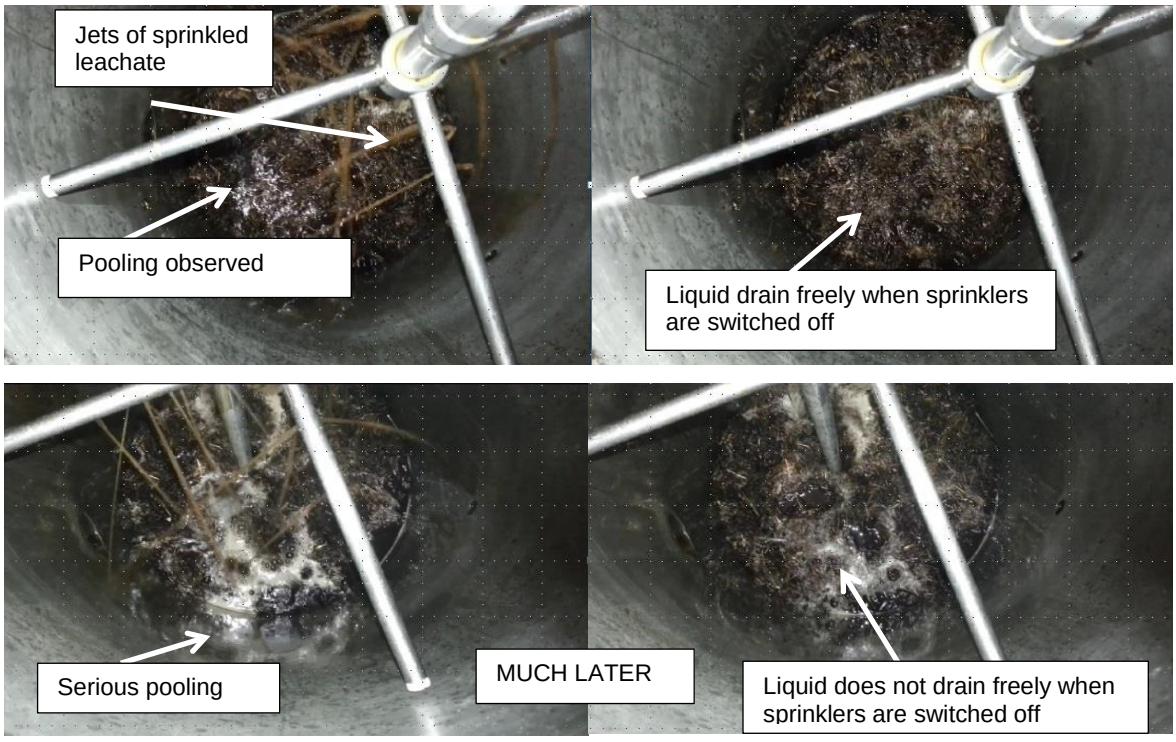

Figure A.3. Leachbed started up with spent wheat straw piggery litter. The photos are grouped in lots of two each representing a period of operation and with liquid being sprinkled over the bed (left) and with the liquid circulation and sprinkling switched off (right).

FigureA.4. (Top) Four-rod sprinkler system hosing leachate over the top of the solid manure residue bed. The leachate is sprayed out of holes in the rods. (Bottom left) Spiral pigtail sprinkler, shown here clogged by straw bedding; (Bottom right) The straw that caused blockage of the sprinkler system. Both the rod and pigtail sprinkler arrangements were used during the prelimianry trials and both experienced severe blockage/clogging.